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HERBERT HICE WHETZEL

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(WITH 3 PHOTOGRAPHS)

In the death of Herbert Hice Whetzel, November 30, at Ithaca, New York, the mycologists and plant pathologists of America have seen the passing of one of their most outstanding figures. He was a distinguished scientist and a vivid, colorful personality. He died at his home on Forest Home Drive, at the head of Beebe Lake, where he had lived for more than thirty-five years beside the campus of Cornell University. For about six weeks he had been too ill to go to his laboratory, and though months earlier at a similar crisis he had made a remarkable recovery, his family and close friends realized that this time the end was near. The insidious malady that had sapped his strength was no longer to be denied. He had fought it courageously, with only occasional mention of the discomfort and pain that he suffered, and his fortitude will remain an inspiration to others who seek attainment against heavy odds. His colleagues in the Department of Plant Pathology will long remember his last years in which, with characteristic stoicism, he tried to ignore his physical condition as he increased his efforts to complete his investigations of the Sclerotiniaceae. He passed away at the age of sixty-seven, in the forty-second year of his days at Cornell. He lies buried at Ithaca in Lake View Cemetery, high on a hillside overlooking Cayuga Lake. The returning former student who seeks out the grave to stand in retrospection, thinking of his old professor, will be rewarded by an

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especially attractive panorama embracing the lake, the valley, and the circling hills. Professor Whetzel is gone. The beautiful countryside about Ithaca will not again see him searching for fungi in sphagnum bog or wooded glen. Yet in nearly every country and clime he lives in the memories of former students who received inspiration from him, respected him deeply, and loved him well.

It is especially fitting that a memorial article concerning Professor Whetzel be printed in *Mycologia*. He served on the editorial board of the journal for seven years, and contributed a considerable number of significant research papers to its pages. At the founding of the Mycological Society of America, in 1931, he was prominent in urging that *Mycologia* be adopted as its official organ of publication. He, more than any other, had favored the formation of the Society and should receive the major credit for taking the initial moves that led to its establishment. He remained actively interested in its affairs and, in 1939, was its president. He attended five of the nine annual summer forays held before the outbreak of the war, and no member collected more industriously and effectively, or contributed more to the success of those occasions than he. At the winter meetings he participated regularly in the sessions of the Society and frequently enlivened its business meeting with a characteristically vigorous presentation of some point about which he felt strongly. As he was long one of America's leading plant pathologists, it is appropriate that a memorial article be published also in *Phytopathology*. This has been prepared jointly by Dr. M. F. Barrus, one of his first students, and Dr. E. C. Stakman, his friend for more than thirty years. Appended to their paper is an approximately complete list of his publications. These number more than two hundred and, as most of them are not mycological in character, duplicate publication of the entire list here in *Mycologia* has seemed undesirable. A personal tribute to Professor Whetzel has been published by Dr. Barrus in the undergraduate journal, the *Cornell Countryman*. An official memorial statement has been prepared by Dr. L. M. Massey, and read by him before the faculty of the New York State College of Agriculture. It emphasizes Professor Whetzel's characteristics and accomplishments, and will be

incorporated by the University in its memorial booklet, "Necrology of the Faculty," which is printed annually for its archives and limited distribution.

Professor Whetzel was born, September 5, 1877, near the village of Avilla, in Noble County, in the northeast corner of Indiana. He was the son of Joseph Conrad Whetzel, born October 31, 1849, in Beaver County, Pennsylvania, and Gertrude (Eckles) Whetzel, born August 4, 1858, in Wood County, Ohio. They were married, October 26, 1876, at Avilla, and had six children, three sons and three daughters. When but a young boy, Joseph Conrad had come to Avilla from Pennsylvania with his parents, Joseph Whetzel and Susanna (Eichling) Whetzel, and had helped his father and brothers clear off timber and establish their farm. At about the same time Gertrude Eckles was brought by her parents from their earlier home in Ohio, where they had married, October 4, 1857. Her father, Valentine Eckles, was born, March 9, 1833, in Holmes County, Ohio, and died, August 12, 1892, in Noble County, Indiana. Her mother, Sarah Ann (Bronson) Eckles, was born in 1837, in Onondaga County, New York, and died, May 13, 1891. They were mainly of Scotch-Irish descent.

The Whetzels came from a line of Pennsylvania "Dutch" farmers. The family had originated in southern Germany, near the Swiss border, and reached America in about 1737. After settling in Maryland, they migrated north into eastern Pennsylvania, and from there some of them took part in the westward movement that occurred about the time of the Revolutionary War. One member of the family, a woodsman and trader, who lived before 1800 in the forest on the frontier near Wheeling, West Virginia, was killed by the Indians. His young sons saw him scalped and their cabin burned. Though accounts differ as to the number and names of his children, one of the sons was the Lewis Whetzel who later became famous as a scout and frontiersman. He swore eternal vengeance on the redmen, and his name is connected with many thrilling episodes of the border warfare. Professor Whetzel claimed descent from a brother of Lewis, and on various occasions alluded with evident satisfaction to this branch of his family tree. The name Whetzel (written earlier Wetzel) had prominence in pioneer days in Indiana also. In 1818, Jacob Whetzel, brother

of Lewis, was living in Franklin County in the southeast corner of the state, a short distance northwest of Cincinnati. Having been granted a tract of land deep in the forest southwest of present-day Indianapolis he set out with his son Cyrus to blaze a trail to their new home. This trail, known in accounts of early Indiana as the Whetzel Trace, began at Somerset (now Laurel) on the White Water River, a tributary of the Ohio, and ran west through the wilderness to the White River, a branch of the Wabash. It ended at the spot now occupied by the village of Waverly. Returning for their families the Whetzels widened the trail, and the next summer drove in with their possessions. Their cabin, erected on the high bluffs overlooking the river, was the first permanent white habitation in Morgan County. Following them, the majority of the early settlers of central Indiana came in over the Whetzel Trace. As Professor Whetzel was pioneering in plant pathology, it pleased him to emphasize that there was much pioneer blood in his veins.

Up to the age of nineteen, his life was spent chiefly on his father's farm in Swan Township, five miles from Avilla. Records filed by him show that he attended the Hopewell country school, and graduated, in June 1895, from Avilla High School. He and a neighbor boy usually walked the five miles to and from school. We may picture him an energetic lad helping with the farm work and, when his chores were done, wandering through meadow, thicket and marsh, interested in the living growing things of nature. Northeastern Indiana is a lovely pastoral region, with attractive woodlands and many small lakes. Stimulated by this environment, the innate curiosity of an observant boy developed into a deep desire to know more about plants and animals. The collector's instinct appeared in him early, and he still treasured, in his later years, a herbarium prepared in his high school days. Even earlier he had accumulated and preserved wild flowers, insects, and fossils. In high school he was fortunate in having in Wallace Harsh a teacher of exceptional ability and understanding, who noted his interest in collecting and encouraged him to go further with his studies. After finishing high school he taught for two years in the local country schools and then, in the autumn of 1897, entered Wabash College at Crawfordsville, Indiana.

Herbert Hice Whetzel
(At about 35 years of age)

Toward the close of his freshman year trouble with his eyes caused him to return home. After staying out of college for a year, when he again taught school, he returned to Wabash in the autumn of 1899 and finished his course, graduating in 1902 with the A.B. degree.

Wabash is a small college of arts and sciences for men. At that period the student body numbered little more than two hundred. The staff consisted of about a dozen professors, all of them very able. In such a school the student is acquainted with most of his fellows and is in close contact with his professors. There Whetzel came under the influence of Professor Mason B. Thomas, an exceptionally successful teacher of botany. In a relatively short lifetime Thomas sent many of his men to the graduate schools of the universities, and saw a goodly number of them attain distinction in botany, bacteriology, forestry, medicine, and plant pathology. Reared in central New York State he had been trained in biology at Cornell, and had gone to Wabash College in 1891 as successor to John M. Coulter. He recognized in Whetzel a student of exceptional promise.

During his four undergraduate years Whetzel, inspired and guided by Thomas, prepared himself for a botanical career. He gained insight into various phases of botany, and seems to have shown early a preference for mycology. In his senior year he presented two small papers before the Indiana Academy of Science, one an experimental investigation of *Gymnosporangium Juniperi-virginianae* (1), the other a taxonomic study of the local species of *Stemonitis* (2). Throughout his life he retained a special interest in the rusts and slime moulds which dated back to those days of collecting around Crawfordsville.

Professor Thomas desired that Whetzel go for graduate work to Cornell, his own alma mater, and most fortunately obtained an assistantship for him there in the Department of Botany under Professor George F. Atkinson. Following his graduation Whetzel proceeded at once to Ithaca, and in early July was already enthusiastically collecting Agaricaceae, Boleti, and other fleshy fungi in the company of Professor Atkinson and C. H. Kauffman. The region about Ithaca with its varied topography and mixture of coniferous and hardwood forest provided in a season of good col-

lecting a rich and varied fungus flora such as Whetzel had not seen before. Throughout that summer he collected energetically by day, and pored over his specimens and the taxonomic literature by night. Years later at the death of Professor Atkinson he wrote in almost a poetic vein of their hours together in the field (**25**). He says: "With large market baskets on our arms we have wandered through the dark damp woods searching eagerly for the choice mushroom treasures that lifted their frail caps from leaf mold, mossy banks, decaying logs or soggy sphagnum. Joyfully we called to each other as we knelt over some rare or gorgeous find. How his eyes sparkled and how tenderly and deftly he lifted the lowly beauty from its place, turning it round before us to admire its slender form or rare coloring. How eagerly we listened to the comments and explanations about it drawn from his wonderful store of knowledge of the habits, haunts and peculiarities of his fungus friend."

At that period Professor Atkinson held the chair of botany in the University, and was also Botanist of the Agricultural Experiment Station. In the latter capacity in preceding years he had emphasized research on plant diseases and had written several well-known station bulletins including one on "Leaf Curl and Plum Pockets" and another on "Damping Off." Of late, however, he had shifted his interests more and more to the study of the mushrooms, and had adopted the policy of referring most of the station work on plant diseases to his assistants. Whetzel applied himself industriously to the problems assigned to him and in April, 1904, published his first station bulletin, bearing the title "Onion Blight" (**4**). It was based on his observations during the preceding season in the fields of commercial growers. He stated it to be a preliminary report, and undertook a more searching study of the disease and its causal organism as a thesis problem for the doctorate degree. In the late spring of 1904, he was promoted to an instructorship with responsibility for conducting most of the plant disease investigations. He remained in that status for two years and, in addition to assisting Professor Atkinson in teaching and research, had nearly completed the requirements of the Graduate School for the degree, when, in the summer of 1906 four years after his arrival at Cornell, he was appointed Assistant Professor of Botany

and head of another Department of Botany then being established by Dean Liberty Hyde Bailey in the newly organized State College of Agriculture. The next year, October 1, 1907, at his own request, his title was changed to Assistant Professor of Plant Pathology, and his department was designated the Department of Plant Pathology. As Dr. Bailey has recently said, "Whetzel broke easily with tradition" and as a result, in this instance, gained the distinction of founding the first department of plant pathology in the United States (Phytopathology **12**: 499). Having accepted appointment to a professorship, he was no longer eligible for his degree at Cornell, and it was never granted, but Wabash College conferred the honorary M.A. on him in 1906 and the honorary D.Sc. in 1931. He had received the honorary D.Sc. in 1926 also from the University of Puerto Rico.

In May, 1904, at his promotion to an instructorship, Whetzel went back to Avilla, Indiana, the home of his boyhood, and married Lucy E. Baker. Returning to Ithaca he spent eight happy years with her in Forest Home. In June, 1912, she was stricken by a baffling illness and died leaving two small children, Gertrude and Joseph, the younger only twenty months old. In the summer of 1914, while abroad on his first sabbatic leave, he married one of Lucy's younger sisters, Bertha A. Baker, in London. Returning home that autumn he began life anew. Twenty-five years later, December 25, 1939, Bertha preceded him in death. Lucy and Bertha were charming and capable women, both much beloved by the department group. Professor Whetzel is survived by his mother, a brother, three sisters, two children and four grandchildren.

Fate was kind, both to plant pathology and Whetzel, in bringing him to Cornell at the beginning of the period of greatest expansion the New York State College of Agriculture has enjoyed. During the decade following the establishment of his department the college appropriations were greatly increased, the staff was much enlarged, and nine of the twelve major buildings now on its campus were erected. He became a full professor at the end of two years, and his department grew rapidly. In the summer of 1907, Donald Reddick was appointed as its first instructor and, after obtaining his Ph.D. in 1909, also was advanced to a full professorship in

First Department of Plant Pathology in its Second Winter, 1908–1909. The entire department group is shown in the south laboratory of the top floor of Stone Hall, Cornell University. Back row—Gertrude Whetzel, H. H. Whetzel, C. N. Jensen, J. J. Taubenhaus, M. F. Barrus, Donald Reddick. Front row—Mrs. Lucy Whetzel, Agnes McAllister, Errett Wallace.

1911. He too had been trained under Thomas and Atkinson. As Whetzel outlined expanding programs of research, teaching, and extension, more positions were made available, and other young men, chiefly from Wabash College, were added to the staff.

During the department's second year, in early January, 1909, the American Phytopathological Society was voted into being at Baltimore. Professor Whetzel and Dr. Reddick were present at that occasion, and both attended the first meeting of the Society the following winter at Boston. At the Boston meeting the Society completed its organization and elected Whetzel a member of its first council. The journal *Phytopathology* was established shortly thereafter. Its first number, printed in Ithaca, appeared in February, 1911, and Reddick was business manager during its first four years. Three editors, L. R. Jones, C. L. Shear, and H. H. Whetzel, with twelve associate editors, constituted the first editorial board. Whetzel served as editor for two years. Reddick passed from the status of business manager to that of editor in 1915, and functioned in that capacity until 1918. Whetzel became president of the Society in 1915, and, during the First World War, served energetically as chairman of its War Emergency Board directing an extensive campaign for improved methods of plant disease control. During the Society's early years the young Department of Plant Pathology at Cornell thus played a prominent and significant role.

Having started with a wholly new department unincumbered by antiquated equipment or impeding precedents, Whetzel showed a keen interest in the problems of organization confronting him and gave much time and thought to details of arrangement and procedure. In Atkinson's laboratories, and probably in those of most departments of biological science at that period, apparatus and materials intended for the general use of staff and graduate students were scattered over desk tops or on open shelves available to all. This lack of order resulted in loss of time and equipment most distasteful to Whetzel. He provided a store room, stocked it very completely, and ruled that staff members and students alike must sign check-out slips at its window. He placed one man in complete control of the department's photographic facilities and had him do all of its photography. Having been adversely im-

pressed by certain inadequacies in the customary method of mounting herbarium specimens of fungi on sheets in genus covers, Whetzel adopted a system in which uniform-sized packets are filed upright in cabinet drawers in numerical sequence without regard to the position of the organisms in the natural classification. Associated materials, such as photographic negatives and prints, slides, and notes, are filed similarly in numerical order in diverse cabinets designed for their respective needs. Accession cards, arranged alphabetically, enable even the most non-mycologically trained to consult the collections. In all such procedures Whetzel sought to provide the best possible working conditions for the entire department group, and though at times in his enthusiasm for organizing he promulgated regulations approaching regimentation his motives in so doing were not selfish, and the measures were designed always for the general good. The department owes its existence and some of its outstanding characteristics to his vision and untiring industry in its early years.

In 1909, confronted with many plant disease problems in New York State which could not be investigated for lack of funds, he committed the department to the then much questioned policy of seeking financial support directly from groups of growers and business organizations. He established "industrial fellowships" under the terms of which the department supplied laboratory equipment and supervision, while those hoping to benefit from the research provided the salary of an investigator (**13**). Each of these fellowships was adequate for the support of a graduate student for several years, and the research undertaken afforded him a thesis problem for the doctorate degree. Located during the growing seasons in a "field laboratory" in the midst of the growers, the fellow conducted his experiments under actual field conditions. The success which attended Professor Whetzel's efforts to obtain funds for the fellowships was phenomenal. In the aggregate they provided many thousands of dollars for endowment of research, increased the department staff, and advertised the institution widely and favorably. He traveled throughout the State, meeting the farmers and addressing their organizations, and at that early period became one of Cornell's best known extension men. He possessed to a pronounced degree the attributes of the success-

ful salesman, and he "sold plant pathology" enthusiastically to the Dean, the grower, and the general public. He kept in close contact with the research in progress in the department, and many of his short early articles on plant disease control represent his efforts to advertise the accomplishments of his students and make their results immediately available to the grower. His personal contribution to phytopathological research is scattered through numerous papers. Many of the investigations conducted by the students were outlined by him and developed under his direct supervision. Though he contributed freely by suggestion and criticism to their success he rarely shared in their publication. He had a contagious enthusiasm for investigational work that infected all who came in contact with him, and his driving energy stimulated the students and staff to increased endeavor.

In 1922, in the fifteenth year of the department's existence, he resigned as its head, and announced his intention to devote the rest of his life to teaching and research. He remained in charge of the elementary courses, while one of his younger colleagues, Dr. L. M. Massey, who had come as a student from Wabash College in 1912, succeeded him as head. Relieved of time-consuming executive duties, Professor Whetzel applied himself to the furtherance of various activities which he had reserved for his later days (Phytopathology **12**: 499).

Throughout his years in the university he had been regarded as an outstanding teacher. His courses had a high reputation among the students; he gave to them unstintingly of his time and energy; and he was a lucid and entertaining lecturer. Nevertheless, he became convinced that in following the methods of teaching in vogue in institutions of higher learning, he was failing to train students to think for themselves, and, after a period of planning and experimentation, he adopted for his elementary course in plant pathology a wholly new method of instruction in which lecturing plays a very minor role. Limited only slightly in his choice, the student selects for study those diseases for which he has a preference, and, the laboratories being open at all times, works at his own convenience, receiving aid from his instructors only when he himself seeks it. Having completed to his own satisfaction the study of one of the diseases, he presents himself

for an individual conference at which he must demonstrate not only a detailed knowledge of the disease, but also the ability to use his facts in the solution of problems, presented for his consideration, in which emphasis is placed on the general principles of plant pathology. This method of instruction is discussed in detail by Professor Whetzel in his article, "An Experiment in Teaching" (**37**). It has proved popular with the students and has given very satisfactory results. Though Whetzel was a teacher of exceptional native ability, his outstanding success in teaching may be said to have resulted in large part from his high evaluation of its importance. He did not subordinate his personal or departmental activities in teaching to those in research, and he looked with favor on the development of well-organized courses as an aid in training graduate students.

Professor Whetzel brought himself to the attention of the younger generation of plant pathologists nowhere more sharply perhaps than in his contributions to the terminology of their science. In his attempt to organize the subject matter of phytopathology for the purposes of teaching he had been impressed by the great need for radical revision of the terminology, and applied himself to the task. He defined the terms precisely, limiting some of the older widely-used ones to a narrower application than that in which they had previously served. Also he coined a number of new terms for concepts not covered with adequate exactness by the older terminology (**35**). Though he encountered resistance to these innovations he used the terms, as he defined them, in teaching and publication, and his students and many others are following in his footsteps to a considerable extent. Probably the future historian of phytopathology will regard Professor Whetzel as one of its great teachers and will emphasize in that connection that his insistence on clear thinking and precise expression was one of his most noteworthy contributions to his science. It must be conceded that in coining new terms, as in some other respects, he tended to be somewhat radical, and clearly took pleasure in nettling the ultraconservatives.

While engaged in his early studies of the diseases of ginseng and peony, Professor Whetzel encountered several species of *Botrytis* and *Sclerotinia* (**17**, **18**, **19**). Obtaining these in pure

culture he made a comparative study of their sclerotia, noting differences in size and shape that seemed to offer a basis for taxonomic separation. Also he became much interested in an early paper in which observations were recorded indicating that *Botrytis cinerea* is the conidial condition of *Sclerotinia Fuckeliana*. In 1913–1914, he spent fifteen months in Europe on sabbatic leave, and, though in residence at the University of Heidelberg during the winter studying plant physiology in the laboratory of Professor Georg Klebs, he found time during the growing seasons for travel and collecting. In the spring of 1914, he searched unsuccessfully on the Continent for apothecia of *S. Fuckeliana* and returned home frustrated in his plan to culture the species and settle the question of its possession of a *Botrytis* stage. A few years later he was especially gratified to be able to coöperate with G. H. Godfrey in demonstrating that *S. Ricini* does unquestionably have *Botrytis* as its conidial condition. These experiences mark the beginnings of his interest in *Sclerotinia*.

As the years passed Professor Whetzel gave an increasing amount of time to research on the genus, and by 1922, when he retired from the headship of the department, had decided to prepare a monograph of the North American species. Each year, thereafter, it was his custom to spend several weeks in the early spring in intensive collecting, searching painstakingly for developing apothecia. His success in finding them was extraordinary. At the time of collection he sought evidence as to their host relationships, and later from additional collections and inoculation experiments verified his assumptions. On his return to the laboratory he obtained each species in pure culture, usually from discharged ascospores. Aided by a succession of technical assistants, trained by him in his methods, he rapidly built up a large collection of cultures and herbarium specimens, accompanied by excellent photographs, drawings, and notes. Soon he had advanced further in the study of these fungi than any preceding investigator, and had available for study in pure culture a considerable number of species never before recorded. Becoming recognized as the authority on the group he received additional interesting material from collectors elsewhere and in 1930 spent eight months in Europe collecting and studying the previously described species of

England, Holland, France, Germany, and the Scandinavian countries in their type localities. Though students of the Discomycetes for the most part had been content to study only the apothecium, he emphasized that many species of *Sclerotinia* cannot be separated on apothecial characters alone. His diagnoses embrace information concerning conidial, spermatial, and sclerotial stages also, and emphasize cultural characters, as well as data on pathogenicity and life-history.

In 1926, he published the first of a series of monographic studies of individual species under the general title, "North American Species of *Sclerotinia*" (**30**). It was not long, however, until he came to realize, from encountering border-line conditions, that his field of investigation must be broadened to include the genus *Ciboria* and other related inoperculate Discomycetes in which the apothecium arises from a stroma or stromatized substratum. Also he became convinced that the taxonomic situation would be clarified by breaking up the older generic concepts into smaller subdivisions. He began calling his group the Ciborioideae, and, in addition to *Sclerotinia* and *Ciboria*, recognized as valid members—*Stromatinia* Boudier, *Monilinia* Honey, *Ovulinia* Weiss, *Lambertella* von Höhnel, and *Rutstroemia* Karsten as emended by Rehm and White. Also he established three new genera of his own, *Septotinia* (**42**), *Martinia* (**47**) and *Coprotinia* (**52**), and had in preparation for publication a paper in which he expected to erect five additional ones. During the last eighteen years of his life he wrote eleven taxonomic papers on the group (**30**, **34**, **41**, **42**, **44**, **47**, **49**, **51**, **52**, **53**, **57**). His last contribution entitled "The Cypericolous and Juncicolous species of *Sclerotinia*" embraces ten species and is expected to appear early in 1946 in *Farlowia*. In March 1943, he proposed the establishment of the new family Sclerotiniaceae (**49**: p. 18) and stated that he would shortly publish a characterization of the family with a synoptical treatment of its genera. This he failed to accomplish. Though he had written a portion of the paper and had prepared a table of contents in which he listed the fifteen genera that he proposed to include, his final illness prevented its completion. He entitled the paper "A Synopsis of the Genera and Species of the Sclerotiniaceae" and expected to condense into it many of his as yet unpub-

lished ideas on family limits, generic separations, comparative morphology, and terminology. He had begun its preparation with reluctance, feeling that such a synoptical presentation could not safely be given until monographic studies of all the genera had been completed. He was aware, however, of the critical condition of his health, and had begun to suspect that he might not live to complete the unfinished monographs.

Though Professor Whetzel in his earlier days had directed most of his investigations toward the solution of economically significant problems in plant pathology, his interests were always largely mycological, and his work on the Sclerotiniaceae during the last twenty-five years of his life was unquestionably his major accomplishment in research. To it he gave his best efforts, and out of it came probably his most noteworthy publications. It is especially to be regretted, therefore, that his untimely death prevented him from summarizing his accomplishments in this important group of fungi.[1]

His enthusiasm for field work was one of his well-known characteristics throughout his life. He collected in Puerto Rico with Dr. E. W. Olive for three months in 1916 (**21**), with Dr. F. D. Kern throughout the summer of 1924 (**28, 29, 31**) and with Carlos E. Chardon in the spring of 1931. He spent the year 1921–1922, on sabbatic leave, in Bermuda and, while acting as the first plant pathologist appointed to the Bermuda Department of Agriculture, found time to collect fungi intensively. He returned to the island in 1926 and, accompanied by Dr. F. J. Seaver and Lawrence Ogilvie, made additional collections (**32, 33**). From the accumulated specimens he prepared the first century of the exsiccati set, Bermuda Fungi. One or more additional centuries are now being prepared, chiefly from these collections, by J. M. Waterston, the present plant pathologist of the island. Finally, in 1939, Whetzel spent three months in Venezuela collecting with Albert S. Müller and Carlos E. Chardon (**40**).

Professor Whetzel was a member of the committee of three, including also Dr. H. C. Cowles and Dr. B. M. Duggar, which

[1] After this memorial article had been submitted for publication, the writer undertook the task of finishing Professor Whetzel's manuscript. The paper is now nearing completion and will appear in an early number of MYCOLOGIA.

organized the International Congress of Plant Sciences (Fourth International Botanical Congress) that met at Ithaca, in August, 1926. He also acted as chairman of the committee on local arrangements and worked indefatigably for weeks to make the congress outstandingly pleasant and successful. The foreign visitors especially will remember his megaphone and his clarion voice sounding through the corridors of Willard Straight Hall as he announced events and gave instructions. Four years later he attended the meetings of the Fifth International Botanical Congress in Cambridge, England.

It has been indicated above that he was a charter member of the Mycological Society of America and the American Phytopathological Society and served as president of both organizations. He was also a member of the Botanical Society of America and the British Mycological Society. Until the last few years he had maintained membership in Société de Pathologie Végétale and Vereinigung für Angewandte Botanik. He was a fellow of the American Association for the Advancement of Science, and an honorary member of the Academy of Medicine of Des Moines, Iowa. He belonged to Phi Delta Theta, Gamma Alpha, Alpha Zeta, Phi Gamma Mu, Phi Kappa Phi, Phi Beta Kappa, and Sigma Xi. The highest local recognition accorded him was his election by the faculty of the whole university to a five-year term as one of their three representatives on its Board of Trustees.

In Ithaca he was widely known outside university circles, and his exceptional capacity for friendship made him one of Cornell's best liked professors. He thoroughly enjoyed his contacts with his fellow men, and conversation and argument meant much to him. He was a member of the Rotary Club and looked forward with anticipation to its weekly luncheon. He was not a sportsman. Games such as tennis or golf made no appeal to him and seemed at best a waste of time. In his hours of relaxation he experienced the greatest satisfaction in working with the soil. During the latter part of his life he developed his home flower garden of nearly an acre until it was one of the most interesting in the community. Though placing little emphasis on arrangement, he assembled many beautiful and unusual plants. His bed of trilliums embraced species from many regions, and he had specialized

in the genus *Sedum* until his plantings attracted even the taxonomist. His extensive and varied rock garden, containing at times as many as four hundred different species, was his special pride. He made a hobby of growing wild flowers from seed, some of them received from former students in distant lands (**48**). In recent summers he contributed occasional articles to the local newspaper in which he announced developments among his plants as the season advanced. The flower lovers of Ithaca were notified when some choice lily was in bloom or were urged to come and enjoy the garden with him at its best.

When this winter's heavy snows have melted away and his trilliums bloom again he will not be there to see. And at the university, in the years ahead, as his former students return, one by one, to visit the old department that holds so much for them of pleasant recollection, the absence of his hearty welcome will be keenly felt. He who was "Prof" to all of them is gone, not to return, but the deeply personal memory of his friendship, his tolerance, and his understanding will be with them as long as they shall live.

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ITHACA, NEW YORK

PUBLICATIONS

An approximately complete list of Professor Whetzel's publications will appear in *Phytopathology*. They number more than two hundred. Many of them deal exclusively with plant disease control, or are short phytopathological notes lacking mycological interest. The list below is believed to include all of his significant mycological contributions, as well as about a dozen papers on other subjects.

1. Notes on apple rusts. Indiana Acad. Sci. **1902**: 255–261.
2. Notes on the genus Stemonitis. Indiana Acad. Sci. **1902**: 261–266.
3. A new method of mounting superficial fungi. Jour. Mycol. **9**: 218–219. 1903.
4. Onion blight. N. Y. (Cornell) Agr. Exp. Sta. Bull. 218: 138–161, *figs. 1–17*. 1904.
5. The diseases of ginseng. Special Crops **4**: 170–171. 1905.
6. The Alternaria blight of ginseng. Cornell Countryman **4**: 33–41. 1906.
7. Some diseases of beans. N. Y. (Cornell) Agr. Exp. Sta. Bull. 239: 198–214, *figs. 100–115*. 1906.
8. A parasitic fungus that winters in the seed of its host. Ontario Nat. Sci. Bull. **4**: 7–9, *figs. 1–4*. 1908.

9. Bean anthracnose. N. Y. (Cornell) Agr. Exp. Sta. Bull. 255: 431–447, *figs. 217–222.* 1908.
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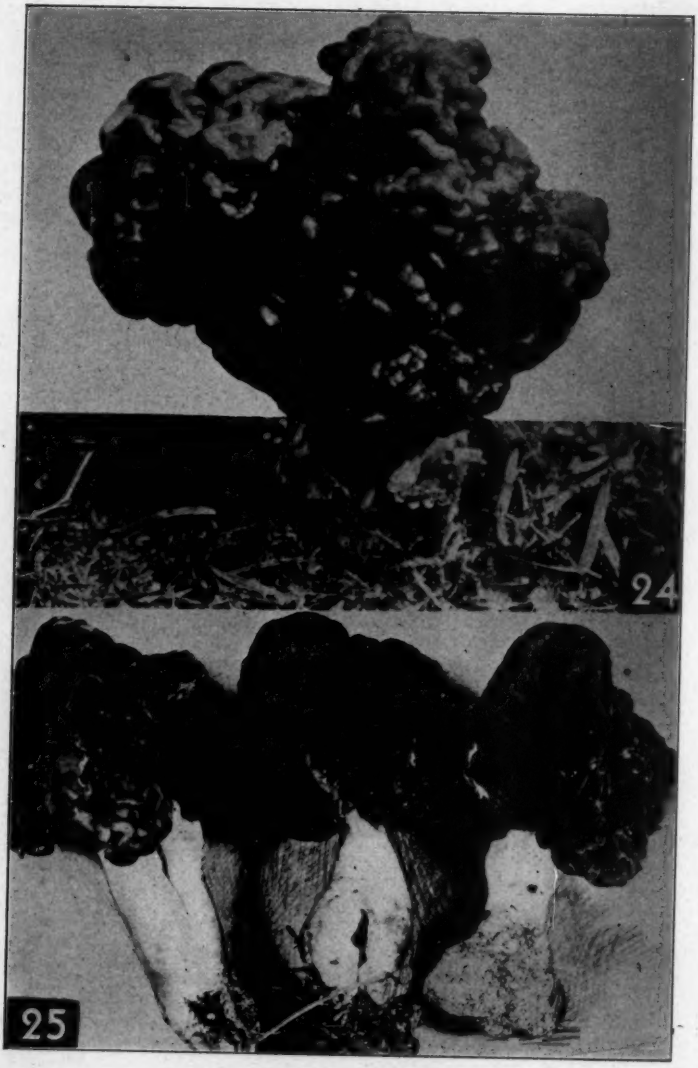


Gyromitra esculenta.

SOME WESTERN DISCOMYCETES GYROMITRA ESCULENTA, HELVELLA LACUNOSA

ELIZABETH EATON MORSE

(WITH 25 FIGURES)

GYROMITRA ESCULENTA FRIES, 1849 [1]

(= *H. esculenta* Pers., 1800) Elias Magnus Fries erected a new genus *Gyromitra* to include those *Helvellae* which have gyrose or brain-like folds in the caps. The two illustrations shown in the frontispiece are believed to be fairly typical of material such as Fries met. In each there is a rather smooth, stout stem at the summit of which is produced an erect, compact head, inflated, knob-like or brain-like in aspect, bearing the hymenium. The two illustrations are quite different, but Fries' brief description applies equally well to each: "pileo inflato difformi undulato gyroso-rugoso brunneo, margine stipiti levi villoso adnexo."

Figure 24 grew in a cold, wet canyon, in disintegrated granite and soil in the lee of a huge log on the northwest slope of Mount Baker, Washington, was photographed and collected by W. T. Shaw, Fresno, California, July 20, 1926. Figure 25 grew in sandy soil in Pacific Grove, Calif., Feb. 24, 1914, N. L. Gardner, collector.

A fine specimen of this species up to eight inches in stature, weighing nearly one pound, was collected by J. Dearness, Ontario, Canada, which he sent to the National Museum of Canada at Ottawa. He reports unfavorably on the edibility of this species, since two fatalities from eating this fungus were investigated by him. Louis C. C. Krieger published this species as "deadly poisonous"; he reported 160 deaths (see Mushroom Handbook, p. 326, 1936). There is a possibility that what we are calling *G. esculenta* is not identical with the European *esculenta.*

[1] See Fries, Syst. Myc. **2**: 16, 17, 607. 1823.

G. esculenta is considered one of the rarest of the large western Discomycetes, in fact the writer knows of no examples other than those shown in the frontispiece which may be claimed to fit into this species as understood.

Whether or not *Gyromitra* should be given full generic rank or be regarded as a variation of *Helvella* is a favorite topic for discussion among mycologists! That *Gyromitra* macroscopically and microscopically is very close to *Helvella* cannot be denied. A structural distinction may be claimed which would secure for *Gyromitra* a standing among genera. Our contact with these gyrose forms is too meager to form an unshaken opinion. On this point, Doctor Dearness writes that he considers that *"Gyromitra* is in the literatures to stay."

For Saccardo's treatment of the four genera *Verpa, Helvella, Gyromitra, Morchella* in equal rank, see his key (Syll. Fung. **8**: 7. 1889).

Of the ten species listed as Gyromitras by Saccardo six of them had been described as species of *Helvella* (Sacc. Syll. Fung. **8**: 15–17, 1889).

HELVELLA LACUNOSA AFZEL, 1783

(= *Elvela Mitra* Linnaeus, 1753)

Helvella lacunosa is a well-known and widely distributed discomycete which has been described and illustrated repeatedly by botanists in many European countries, in Canada and in the United States. It is an exceedingly variable species in stature, form, coloration and general aspect. In all localities with the exception of the Pacific Slope an estimate of average stature is 6–7 cm. Specimens in the region of Seattle, Washington, have reached 20 cm. in height (Stuntz), in Berkeley 16 cm. (Morse), and in Santa Barbara 18 cm., and cap 5–10 cm. broad (P. M. Rea). Thus far the range is from Santa Barbara, California to Pitt Island on the coast of British Columbia, found in deep moss, under *Thuja plicata,* rainfall approximately 100 inches (McCabe); doubtless this range will be extended both north and south.

The main purpose of this article is to present variations which occur in this western area. These variations may be attributed

wholly or in part to great humidity and to the mild two-season climate of the Pacific Slope.

My favorite collecting ground is a steep hillside planted to *Pinus radiata* and *P. edulis,* overlooking the stadium of the University of California in Berkeley with the Golden Gate in the distance. Specimens arise in dense colonies from soil beneath a carpet of rotting coniferous duff. They appear during and following the heavy rains in the autumn and winter months, with temperature about 60 to 70 degrees. The steep hillside is significant, because it affords excellent drainage and helps to prevent the decay of fungous tissue.

The description which follows is based upon the observation and study of a large amount of material, both fresh and dried, and may be considered composite.

HELVELLA LACUNOSA AFZEL

Pileus—up to 9.5 cm. wide, blackish from the first, brittle, wax-like in texture, mitrate, inflated, variously folded and contorted, borne on ribs which are extensions of the cortex of stipe, turns back on, or is attached to, the stipe at several points, grayish on under side.

Stipe—up to 20 cm. long by 3.5 cm. wide, snow-white at first, later becomes smoke-gray, wax-like in aspect but cartilaginous, often labyrinthine throughout (FIG. 7), passages often closed making pockets, or opening to the exterior showing slit-like apertures of great variability; may be fairly slender, equal, or ventricose and narrowed to base, or much enlarged at base (FIG. 4); cross sections of stipes show honeycomb aspect of empty lacunae (no liquid) (FIG. 7); stipes may be very wide (FIG. 10), or two stipes may be completely coalesced (FIG. 8), or partly so, or merely attached at bases by mycelium; abundant mycelium may bind gravel and soil into solid masses, at base of stipes, usually left by collectors in the forest duff (FIGS. 12, 16, 17; also Mycologia **35**: 574–5, *figs. 10, 11*).

Asci—cylindric, gradually narrowed to base, around 275 × 15 μ, 8-spored (FIG. 23).

Spores—ellipsoid, hyaline, smooth, containing one large oil-drop, 19–22.5 × 12.5 μ, uniseriate.

Paraphyses—septate, slightly enlarged at tips.

A structural distinction in the arrangement of the fertile tissues of *Gyromitra esculenta* and *Helvella lacunosa* may be claimed: as

previously stated, *Gyromitra* makes a definite, erect, compact head on a stem stout enough to support it, while *Helvella* grows a cap with lobed margins which turn back on the stem to which it is usually attached at intervals.

There may be a crumpling of the fertile tissues of the cap which suggests relationship to *Gyromitra,* but no specimens ever extend beyond the crumpled stage; furthermore, the peculiar distinctive lacunose stipe which characterizes this species holds these larger specimens in this series (FIGS. 6, 7, 8, 9, 10, 11).

Various types of lacunose stipes are met in *Gyromitra,* but we are not aware of any like these shown in our illustrations of *Helvellae.* I consider the *Gyromitrae* shown in the frontispiece distinctive and unlike all my other collections.

ANTIQUITY OF THE NAME HELVELLA

Referring to the voluminous notes supplied me by J. Dearness, we find that the word *Helvella* may have come from a Greek word allied to our "edible" in meaning. If the Greek spelling begins with " the syllable is aspirated, *e.g.* *h*-e-l-v; if it begins with ", the syllable is smooth. Greek dictionaries place both forms in the same column. The first letter of a word is important in indexing.

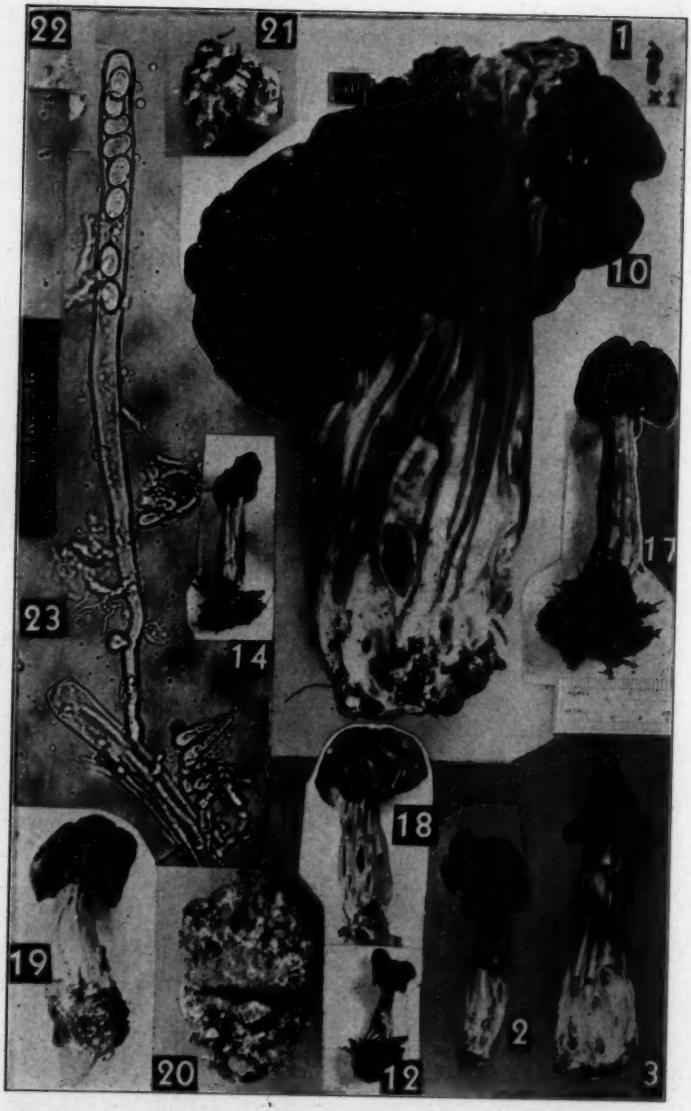
Cicero, born 106 B.C., died 43 B.C., and other Latin authors, used the word *Helvella,* applying it to a kind of fungus.

Coming to the time of Linnaeus, he, in 1753, appropriated the name for a genus, spelling it *Elvela;* however, he changed that spelling two years later to read *Elvella.* Eight years later, in 1763, in his second edition of Species Plantarum he described *Helvella Mitra,* implying a choice and decision of spelling which we are adopting.

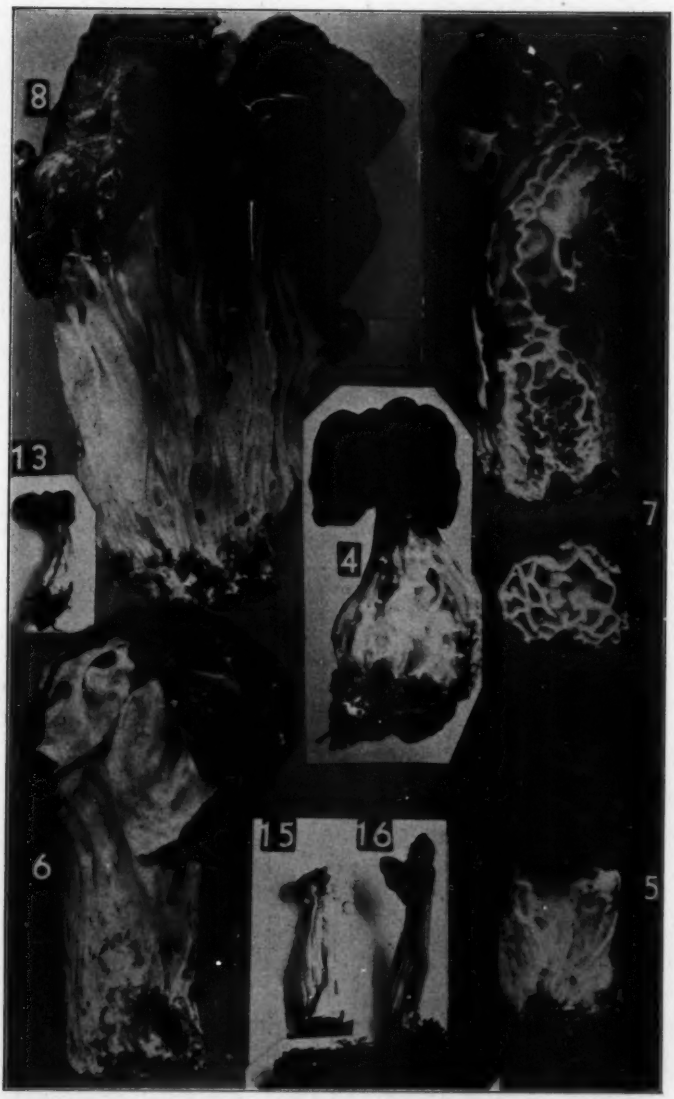
In regard to the use of "Mitra" as a species name, Dr. J. C. Loudon, a good Latin scholar, in 1865 states that "Mitra" has been applied to so many species that it, *Mitra,* has been abandoned altogether. Many expert mycologists have referred *H. Mitra* to synonymy of *H. lacunosa.* Dr. Seaver in the additions to his monumental work [2] states that *"Mitra"* should be replaced by *"lacunosa"* Afz.

[2] *The North American Cup-Fungi (Operculates),* 1942.

Helvella lacunosa.



Helvella lacunosa.



Helvella lacunosa.

Adam Afzel was a pupil of Linnaeus at the University of Upsala. He described *Helvella lacunosa* in 1783; this name was accepted by Fries forty years later, and thus both the genus and species names became "legal," and in accord with the International Rules of Nomenclature.

FRIES' DESCRIPTION OF HELVELLA LACUNOSA

Syst. Myc. 2: 15. 1823: "pileo inflato lobato, cinereo-nigro, lobis deflexis adnatis; stipite fistuloso, costato-lacunoso."

We are not inclined to apply his varieties "major" and "minor" to our material, since he describes larger plants as having white stems, and smaller with smoky stems. All our specimens have stems more or less "smoky," according to climatic conditions. The transition from small to large is gradual, and no dividing line can be set. Also, we do not use var. *fumosa* Ellis & Ev. (North Am. Fungi *3039*. 1896) because smokiness of the stipe had been noted by Fries. At least six other varieties in addition to the above have been described by authors. These are merely speculative, as far as I know. I would not use them.

EDIBILITY OF H. LACUNOSA

Several authors claim edibility as a feature of this species. Odell, associate of Gussow, Canadian mycologist, was interested in fungi mainly from the standpoint of esculence; he states: "fried in butter, they make a delicious dish." Collector Perona, Sausalito, California, eats this species, but he does not find it uniformly desirable from season to season. Most authors are silent on this point.

DISCHARGE OF SPORES WITH AUDIBLE SOUND [3]

If several specimens of ripe *H. lacunosa* are placed in a container, *e.g.* a shoebox, for several hours, and the cover then removed, the currents of air cause the lids of asci to fly up and millions of spores are released at the same moment. The discharge is accompanied by a distinct, suppressed, hissing sound. This is not an isolated experience with us. See A. H. Reginald

[3] For a popular account see The Whispering Fungus, Nature Magazine, August 1934, p. 84.

Buller, Researches in Fungi, Vol. I, p. 258, for discharge of spores in *Peziza Acetabulum* and *Helvella crispa;* also De Bary, Comparative Morphology and Physiology of Fungi, 1887, p. 92.

ASSOCIATION WITH TRICHOLOMA SCLEROTOIDEUM MORSE

Mycologie **35**: 573–581. 1943

There is no more recent report to make. Doctor Dearness hopes the question of relationship will yet be answered, and closes with the query: is the association accidental, symbiotic, or parasitic to the *Tricholoma* and to the *Helvella?*

It is my personal opinion that the strange, cheese-like growths out of which the agaric arises, in some way not understood, originate from the mycelium of the abundant *Helvella lacunosa* growing on the same hillside.

Doctor Dearness has been making studies on *H. lacunosa* since 1917, in Swedish, Swiss, German, French and English literatures, and has supplied these to me together with tracings from colored and black and white plates, microscopic measurements, and personal impressions. I am wishing to make grateful acknowledgments to him, to Doctors Lee Bonar and F. J. Seaver, as also to our technical assistant, Mrs. V. P. Miller.

CALIFORNIA MYCOLOGICAL SOCIETY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA,
FEBRUARY 1, 1945

EXPLANATION OF FIGURES

Photomicrograph and photographs by W. C. Matthews and Victor Duran unless otherwise stated.

Helvella lacunosa Afzel.

Fig. **1–11, 20–22** (× 1, or slightly reduced) (Morse). All caps black, all stipes lacunose, more or less "smoky." Fig. **1**, young ascophore, cap and stem differentiated; **2**, fully formed, cap lobed, free, stem equal, lacunose, orifices pronounced; **3**, cap saddle-shaped, stem enlarged at base; **4**, stem much enlarged at base, cap mitrate, margin lobed, free; **5**, a transitional form, cap expanded, shows *Gyromitra* aspect; **6**, cap contorted, lobed, two lobes turned up show grayish under-surface; **7**, vertical median section shows structure in attachment of cap to stipe, cap turns down on sides of stem (*Gyromitra* makes a head at summit of stem), lacunae branch and coalesce, often open to exterior; cross section shows lacunae vary in size and shape, dry; **8**, two stems completely coalesced or one broad stem branched

at summit; pale area at left cap shows ochre coloration; 9,[4] a perfect, typical, well balanced specimen, attains the "high-water mark" of this species; cap lobed, free as in figure 4; white spores lodged in depressions of cap; smokiness of stem is confined to the epiderm; stem fluted, orifices large (cross section from another specimen); 10, also a prize specimen, "stocky," cap lobed, convolutions not far removed from some *Gyromitrae;* 11, cap, saddle-shaped to lobed (Fig. 3), grown to stipe on under side, somewhat convoluted, but never attains the brain-like aspect of *G. esculenta;* shows the maximum stature in this series.

Fig. 12–17 (× ½), Richardson collection, Eureka, California, Swanlund studios; 12, 13, 14, 15, 16, caps saddle-shaped; 15, attacked by mold, *Mycogone cervina* (determined Cash, Washington, D. C.); 17, cap mitrate, lobed, attached at intervals to stipe.

Fig. 18, 19, from Setchell-Gardner collections, Marin Co., also Pacific Grove, Calif. Caps mitrate, free, convoluted, stems typically lacunose; reduced.

Fig. 20, 21, 22, solid, basal masses of soil and gravel bound firmly by mycelium (no "cheese"), not readily broken apart. (See Mycologia 35: 574, 575, figs. 10, 11, 1943.) See scale in margin.

Fig. 23, photomicrograph of ascus (280 μ, J.D.), narrowed below, lodged against another ascus, eight spores towards the tip, walls smooth, each spore containing one large oil-drop.

CONCLUSION: I am inclined to think that large forms such as I show at figures 6, 7, 8, 9, 10, 11 may not have been met often by other collectors. Doubtless Schaeffer (1774) had large forms, for he described a variety "major" (Sacc. 8: 19).

We have here in the west many notable examples of giant forms in other species of fungi. It appears that *Helvella lacunosa* extends that list.

[4] For a companion specimen, see Textbook of general botany, Holman and Robbins, fig. 307, p. 411, 1934.

NEW AND INTERESTING SPECIES OF BASIDIOMYCETES

R. SINGER

(WITH 1 FIGURE)

The following notes are intended to present new facts on various groups of Basidiomycetes, especially Agaricales. It is, no doubt, preferable to treat the taxonomic groups separately and monographically, but in many instances, the information available is either too incomplete to be combined into monographic studies, or comes merely as a more detailed supplement to be added to existing monographs. If such scattered information were withheld, the progress of taxonomic mycology would indeed be much slower. This first series of new and interesting species is concerned mainly with material from Florida.

I. A NEW SPECIES OF CLAVARIA

Clavaria floridana Sing. sp. nov.

Carpophoris caespitosissimis sed vix base connatis quamquam saepe fasciculariter crescentibus, simplicibus vel bifurcatis in parte inferiore vel saepius in parte superiore, rarissime ramosis, saepissime compressis vel canaliculatis, apice acuto et parte superiore fertili cinerea (in vivis), exsiccando atrocinerea instructo, parte inferiore abrupte delimitata, albo-flavida, tenuiore quam pars superior fertilis, 50–80 × 2–5 mm., intus albis, carnosis, moderate fragilibus, inodoris, mitibus. In dumetis tropicalibus ad terram.—Sporis levibus, 7–8.5 × 5.8–7.5 μ; cystidiis et fibulis nullis; basidiis bisporis.

Carpophores very cespitose (from afar sometimes like a gray turf) but the bases not connate though often fasciculate, simple or forked below or more often above, rarely almost crested as in *Clavulina cristata*, very rarely 2- to 6-branched, very frequently compressed, or canaliculate, with acute tips, the larger upper fertile part cinereous when fresh, dark cinereous when dried, the lower part sterile, yellowish white or whitish yellow, yellowish when dried, the fertile and the sterile parts very abruptly and distinctly delimited, mostly about 2–3 mm. broad in the fertile part, rarely up to 5 mm. in diameter, 50–65 mm. high, more rarely up to 80

mm. high; context white, fleshy, moderately fragile, solid, inodorous and mild to the taste. Spores 7–8.5 × 5.8–7.5 μ, subhyaline, subglobose or very shortly ellipsoid, with a large central oil-drop, non-amyloid, smooth; basidia 38–42 × 6.7–7.5 μ, 2-spored; cystidia none; trama hyaline, subregular, but the hyphae strongly interwoven, very variable in size and shape, short to long, attenuate at the septa or equal, 2–15 μ in diameter, thin-walled; clamp connections none; pigment a membrana-pigment, olive, brownish-melleous in alkalis.

Habitat: In tropical hammocks, usually on the ground or occasionally on small rotten sticks or leaves, fruiting in summer and fall, Dade Co., Fla. (type collection *R. Singer F 733,* preserved at the Farlow Herbarium).

This is not like any of the northern species. It reminds one remotely of *Clavaria cinereoatra* Rick from Brazil which, however, has larger spores (in our specimens, they are 8.5–10.3 × 7.5–8.8 μ). The distinction between *Clavulina* and *Clavaria* as pointed out by Donk seems rather satisfactory on paper but the more species are examined the more obscure it becomes. Even the cytological distinction is uncertain since only a minority of the species have been studied in this regard. In my experience, the typical *Clavulinae* have clamp-bearing septa. This would tend to exclude *C. floridana* from the genus *Clavulina,* and its basidia may be expected to be chiastic.

II. A LACTARIUS WITH YELLOW, WATERY LATEX

Lactarius xanthydrorheus Sing. sp. nov.

Pileo isabellino, magis brunneolo discum versus vel centro intensius colorato vel colore *Marasmii floridani* Murr. gaudente, marginem versus saepe ad olivaceum vergente, margine saepe subsulcato-subcrenato, centro plerumque distincte rugoso-venoso, subglabro, subplano, margine deflexo, disco papillato, dein omnino plano (papilla neglecta), demum concavo, 9–25 mm. lato; cuticula structura *Russulae virescentis* gaudente.—Lamellis cremeis, subdistantibus, arcuato-decurrentibus in tertia interiore, latiusculis; sporis in cumulo cremeo-albis; sub microscopio 8.7–10 × 7.5–8.7 μ, cystidiis sparsis.—Stipite lamellis concolori versus apicem, media in parte lamellis vel pileo concolori, ad basin albido, solido, dein cavo, subglabro, levi, 11–22 × 3 mm.—Carne alba vel albida, fragili, miti, inodora; latice aquoso, pellucido, luteo. In silvis et dumetis humidis.

Pileus "Isabella color," sometimes more brownish on the papilla, or deeper and richer colored in the center, or approaching the color of *Marasmius floridanus* Murr. (this color cannot be matched

in the color charts), often tending to olive on the margin (*pl. 15, L 12,* or *pl. 14, L 9,* Maerz & Paul), often short-sulcate and almost crenate at the margin, the center usually conspicuously rugose-venose, rarely less conspicuously so, subglabrous, almost flat with initially convex-deflexed margin and papillate center, very rarely an occasional individual without papilla, the papilla persistent in age, even in the last concave stage, 9–25 mm. broad.—Lamellae cremeous (*pl. 9, C 2, M. & P.*), subdistant, *i.e.* 19–27 through-lamellae present, subventricose near the margin and arcuate-decurrent behind, rather broad (about 5 mm.); spore print creamy white (between A and B of Crawshay).—Stipe at the apex concolorous with the lamellae, in the middle also concolorous with the lamellae or concolorous with the pileus, at base whitish from a tomentose mycelioid coating, subglabrous, smooth, solid, sooner or later becoming hollow, mostly subequal, 11–22 mm. long and about 3 mm. broad.—Context of the pileus white, of the stipe sordid white, fragile in all parts; odor none; taste mild. Latex not milky, watery and transparent, yellow.

Microscopical characters: Spores 8.7–10 × 7.5–8.7 μ, short-ellipsoid to subglobose, echinate, asymmetrical, hyaline, ornamentation 1–1.8 μ high, consisting of spines or short ridges, connected by low veins forming a complete or incomplete network (type IIIa, IIIb, or II, few IV, V, VI); basidia 42–49 × 9–10.5 μ, clavate, 4-spored; cystidia about 55 × 7.5 μ, very few, without banded contents, subfusoid-clavate, hyaline; cuticle of the pileus consisting of chains of spherocysts (8.5–23 μ in diameter) with filamentous terminal appendages, the latter forming the epicutis, 22–25 × 4–7 μ, sometimes not separated from the last spherocyst by a septum, and then the last member bottle-shaped, all these elements filled with a brownish-fuscous cell-sap; all septa without clamp-connections.

Chemical characters: **KOH** on surface of pileus, negative; with latex "primulin yellow" to "light cadmium" (*i.e* more intensely yellow).—**NH_3, NH_4OH, HNO_3** on surface of pileus, negative.—**$FeSO_4$** on surface of pileus, negative; on context "normal" (*i.e.* reddish gray).—**Phenol** deep chocolate.—**Methylparamidophenol** on context indistinctly reacting, strongly positive only with the edges of the lamellae.

Habitat: In dense low hammocks, especially when intermixed with *Pinus palustris,* on the soil, along the trails, on very decayed stumps, among *Sphagnum* or other mosses, or among pine needles; gregarious. Fruiting in July and August.

Distribution: Florida.

The type (*R. Singer, F 134*) from Highlands Hammock State Park, and co-types from there as well as from the Sugarfoot Hammock, Alachua Co., Fla., is preserved at the Farlow Herbarium. This species belongs in the section *Plinthogali* Burl. because of the structure of the cuticle, but it is most unusual in regard of the latex. The latter is possibly at first hyaline but changes to yellow so fast that the hyaline stage cannot be observed.

III. A NEW SPECIES OF RUSSULA (FIG. 1)

Russula ferrotincta Sing. sp. nov.

Pileo, albo, pallide lilaceo tincto vel pallide lilaceo et albo-maculato, maculis ferrugineis minutissimis saepe asperso, cuticula sicca, subvelutina vel velutina margineque obtuso, at haud rotundato, levi praedito, convexo, applanato centroque depresso in vetustis, 50–131 mm. lato; epicute corpusculis laticiferis destituta, crinibus microscopicis obsita.—Lamellis candidis, interdum fractis brunnescentibus, aequalibus vel nonnullis brevioribus intermixtis, angustis, anguste adnexis, subconfertis vel confertissimis; sporis in cumulo albis; sporis 7–8 × 5.5–5.8 μ, verrucis venulis subconnexis, 0.2–0.3 μ altis obtectis; cystidiis sulphovanillini ope caerulescentibus.—Stipite candido, subtiliter pruinoso, e solido farcto, aequali vel basin versus attenuato, 45–132 × 15–34 mm.—Carne alba; odore nullo; sapore miti; $FeSO_4$ varie reagente, solutione phenolica lilascente. In silvis sub quercubus, vernalis.

Pileus white with more or less extensive "light purplish vinaceous" or "pale purplish vinaceous," more rarely "pale brownish vinaceous" areas which eventually become more or less "brownish drab," sometimes with this lilac tinge occupying the larger part of the pileus while in other caps not a trace of it is seen, but most caps in between these extremes, with the lilac color more often concentrated near the margin than otherwise, dry, subvelutinous to velutinous, less velutinous toward the center, frequently faintly rivulose, often cracking rimosely on the disc, opaque, often with some rutsy spots or dots, especially near the margin, convex, then with depressed center, the marginal half eventually becoming flat, with the margin itself always obtuse but not rounded (subobtuse), smooth, only in some very old and large specimens eventually sulcate, 50–131 mm. broad, usually 75–95 mm. broad.—Lamellae white, sometimes stained brown where wounded, a few or many forked, with very few to many lamellulae, not broad (4–5 mm.), or sometimes broader in caps of more than 100 mm. diameter, not ventricose, subclose to crowded, broadest in the marginal third, rather flexible (less so than in *R. cyanoxantha*) to moderately brittle, narrowly adnexed, often with decurrent tooth; spore print pure white (A in Crawshay).—Stipe pure white, exceptionally

with a "pale purplish vinaceous" hue at the apex (but probably from pigment washed off the cuticle of the pileus), finely pruinose all over, solid and firm, becoming spongiose and fragile with age, equal or tapering downwards, 45–132 × 15–34 mm.—Context white, rather firm at least initially, odor none; taste mild.

FIG. 1. *Russula ferrotincta.*

Microscopical characters: Spores (from print) 7–8 × 5.5–5.8 μ, ellipsoid, or nearly subglobose, hyaline, warty, ornamentation of type IIIb or IV, rarely V (*i.e.* warts connected by a few to many thin lines which may form an incomplete network), 0.2–0.3 μ high; basidia 34–44 × 8.7–10.2 μ, 4-spored; cystidia 35–58 × 7.8–10 μ, versiform, mostly clavate-subfusoid, bluing at least in the upper half in sulphovanilline; trama with rather numerous spherocysts; epicutis of the pileus consisting of erect hyaline, non-incrusted hairs; hairs capitate, clavate, ventricose below or in the middle and then ampullaceous above, etc., (8)–20–50 × 4.5–7.5 μ, forming a palisade; the basal hyphae of these hairs erect or ascendant, usually short, narrowed at the septa (thus sometimes approaching the shape of a spherocyst), forming short chains which constitute the subcutis.

Chemical characters: **KOH** negative.—**$FeSO_4$** on surface of pileus, gray to slate violet or greenish (to "dark olive buff"), surprisingly variable in different collections but always positive and in the above colors; on context of stipe salmoneous or salmoneous-pallid, or greenish in the cortex, gray in the cottony interior (very variable); on lamellae green or yellowish cinnamon with a salmoneous tinge, or salmon color.—**Sulphovanilline** negative or merely blue with some purplish when applied in fresh condition; when applied in dried condition, it causes no reaction or a deep blue one in the cottony interior of the stipe, and a bluish black and carmine one in the cortex, or becoming "garnet brown" all over.—**H_2SO_4** on surface of pileus, duller flesh color (reaching color of *R. vesca*), then bleaching to white.—**Phenol** in cottony interior of stipe "normal" (to chocolate color), but in cortex "deep brownish vinaceous," "russet vinaceous," "sorghum brown," eventually deep chestnut with the cortex remaining as above.—**Methylparamidophenol** positive (violet), moderately strong and moderately fast reaction.

Habitat: In high hammocks with *Quercus virginiana* on the ground, in small groups.

Distribution: In and around Gainesville, Alachua Co., Florida, U. S. A.

The type is preserved in the Farlow Herbarium (*F 1889 a*); other collections (*F 1889, F 1889 b, F 1889 c, F 1889 d, F 2103, 2103 a*) are co-types. Our figure 1 is based on additional material collected later in June.

This species reminds one somewhat of *R. cyanoxantha* but it has at least a subvelutinous pileus, not to mention the anatomical and chemical differences. It belongs in the section Rigidae where it is somewhat intermediate between the Lilacea-group of the subsection Lepidinae and the Vesca-group of the subsection Chlorinae. I have compared all of Murrill's types at Gainesville but neither the specimens nor their descriptions of any fit this new species. It reminds one most of the northern *R. flocculosa* Burl. The type of the latter has been examined by the writer (Mycologia **34**: 75. 1942) and found to be similar to *R. vesca* in many regards, especially as far as the structure of the epicutis is concerned. It is precisely the structure of this layer of the cuticle that makes it safe to assume that *R. ferrotincta* is different from *R. flocculosa* because the hairs of the latter are much narrower as compared

with the basal cells, and more filamentous. Also, the lamellae are subdistant in *R. flocculosa* instead of close.

IV. REDESCRIPTION OF RUSSULA PULVERULENTA PECK

Pileus between "drab" and "buffy brown," nearer to the latter in most cases, or with more pale buff or grayish pallid throughout, initially pulverulent all over with a loose mealy velar covering and fine isolated flocculae around the margin, this veil about "amber yellow" and easily rubbed off the cuticle proper (never innate), the pileus becoming glabrescent after strong rains and in age and then much like *Russula pectiata,* minutely radiately rugulose, somewhat viscid after rains, with separable cuticle (excepting the depressed part of the pileus), with a 6–10 mm. broad marginal zone strongly pectinate-sulcate at maturity, with acute margin, subsemiglobose and often somewhat umbilicate, becoming convex and usually depressed in the center, eventually often concave, 42–80 mm. broad.—Lamellae white to whitish, eventually pale cream color, rather narrow to medium broad (3.5–8 mm.), attenuate-free, close to medium close, not ventricose, anastomosing or not, forked at the stipe, equal or irregularly intermixed, simple or with many forked ones intermixed, broadest in the middle; spore print pale cream color (B or Crawshay).—Stipe white, somewhat fulvous-yellow or yellow on the base (from veil), glabrous to subglabrous, subrugulose, equal or subfusoid, soft, soon becoming hollow, 25–54 × 8–20 mm.—Context white, often somewhat sordid-gray under the cuticle of the pileus, rather fragile, at least when old; taste submild but somewhat disagreeable to acrid, its acridity up to moderate; odor oily (like that of *R. foetens* but lacking the almond or nitro-benzine element), but weak.

Microscopical characters: Spores, basidia, cystidia, and cuticular elements see in my analysis of Kauffman's material (Bull. Soc. Myc. Fr. **55**: 230. 1939). The cuticle is not visibly divided into an epicutis and a subcutis, the epicutis proper being reduced to occasional hair-like terminal members of the subcuticular hyphae; the veil, if examined in fresh material (otherwise it is almost impossible to find it and examine it separately), consists of slender, tender, elongate, cylindric-filamentous, smooth hyphae with cylindric terminal members, with rounded tip, with thin walls, clampless septa, hyaline to ochraceous (in NH_4OH) cell-sap and 2.5–3.5 μ diameter; neither the veil nor the cuticle proper contain elements bluing in sulfovanilline, even when fresh.

Chemical characters: **KOH** with the vein on the pileus and on the base of the stipe immediately and characteristically "Mars orange"; with the cuticle slightly darkening; on context somewhat

vitreous-hyaline-gray.—**HNO_3** on veil and cuticle, negative.—**$FeSO_4$** on context, pale reddish gray (dirty salmoneous with some yellowish gray mixed in), so-called "normal" reaction.—**Chlorovanilline** negative, eventually becoming blue.—**Phenol** on context, fast rather rapidly changing to chocolate color.—**Methylaparamidophenol**: quickly and strongly dark violet in all parts.

Habitat: In hammocks, mixed and frondose forests, on shaded lawns and in gardens, on earth or on decayed wood (*e.g. Liquidambar styraciflua*) or on the base of frondose trunks (*e.g. Persea*), most frequently found in the neighborhood of oaks but by no means exclusively with *Quercus*.[1] Mostly solitary or in small groups; fruiting from May until November in Florida, comparatively shorter fruiting periods are observed farther north.

Distribution: From New England west to Michigan and south to the southern tip of Florida and along the Gulf Coast, becoming increasingly common toward the south.

Several interesting conclusions can be made from the above data. The veil is the same as in *R. subvelata* Sing. from the Caucasus, type species of the section Subvelatae, and, except for a slight difference in color, in *R. mutabilis* Murr. from Florida. This veil has the same appearance and anatomical structure as in the pulverulent boletes (*Pulveroboletus Ravenelii* and *P. subacidus*). *R. pulverulenta* differs from *R. subvelata* chiefly in the not white (A) but pale cream (B) spore color, the either disagreeable or acrid taste and oily odor (though both odor and taste are rather weak), the slightly stronger development of the velar layer in the center of the pileus in an average of young specimens, and perhaps the slightly smaller number of hair-ends in cuticular hyphae. While *R. pulverulenta* prefers oaks, though often growing independent of them, *R. subvelata* seems to prefer *Carpinus*, yet sometimes is found far from them. The taste is occasionally mild in *R. pulverulenta*, and then the two species may be rather similar in their external characters. Peck's type is the same species that we have collected and described above. However, Beardslee's *R. pulverulenta* is definitely something else. We had difficulties in finding the proper position of this species in the classification

[1] In one case we collected it with *Pithecolobium* and fruit trees around it but all the usual mycrorrhizal trees completely absent.

mainly because of this difference in interpretation in the American literature. We thought that it may possibly belong in the section Subvelatae (Bull. Soc. Myc. Fr. **51**: 303. 1935) but later transferred it to the Ingratae (Bull. Soc. Myc. Fr. **55**: 230. 1939). Both these sections are more closely related to each other than to any other sections of *Russula*, yet, as far as *R. pulverulenta* is concerned, it turns out that our first guess was right.

V. A NEW SECTION AND A NEW SPECIES OF TRICHOLOMA

While discussing certain types of species belonging to *Tricholoma* and allied groups (Lloydia **5**: 111–117. 1942), we have, in a sketchy way, outlined our classification of the tricholomas proper. This new classification can be expressed in the following key:

A. Clamp connections present; surface of the pileus not sericeous; odor not of gas tar or lilac flowers.....Subgenus **Contextocutis** Sing. nom. nov. (*Rigida* Fr. *sensu orig.*)
  B. Pigmentless species.....Section *Leuco-Rigida* Sing. *ined.*
  B. Pigmented species.
    C. Spores nearly evenly ellipsoid to ovoid.....Section *Rigida* Fr. em.
    C. Spores subangular, crest-shaped, etc.....Section *Io-Rigida* Sing.
A. Clamp connections rarely present, and then the pileus at least subsericeous, whitish, and with odor of gas tar or lilac flowers.
Subgenus *Eu-Tricholoma* Lange, em.
  D. Hyphae of the cuticle of the pileus subparallel, never gelatinized; pileus sericeous or subglabrous with initially fibrillose-velutinous margin; odor of gas tar or lilac flowers, more rarely absent.
Sect. *Sericella* Fr. em.
  D. Hyphae strictly parallel at least in the upper layer of the cuticle, or if somewhat wavy, distinctly imbedded in a gelatinous mass; pileus viscid and then often glabrous or innately fibrillose, or dry and then mostly distinctly tomentose to squamose, not sericeous.
    E. Pileus gray, umber, whitish with gray fibrils, or golden lemon yellow; lamellae white, yellowish, gray, or pink, not rusty-spotted.
Sect. *Limacina* Fr. em.
    E. Pileus cinnamon, buff, orange yellow, orange red, rufous-brown; lamellae white, buffy pallid, pallid, light yellow, often with rusty spots, especially when old.....Sect. *Genuina* Fr. em.

The section *Io-Rigida* Sing.[2] consists of several interesting and rare species one of which does not seem to have ever been de-

[2] *Tricholoma*, subgen. **Contextocutis** nom. nov. (= sect. *Rigida* Fr. *Tricholomate saponaceo* Fr. typo), sect. **Io-Rigida** Sing. sect. nov. Sporis subangulatis vel cruciformibus; hyphis fibuligeris; pigmento saepe lilascente vel purpurascente. Species typica: *Tricholoma pseudosordidum* Sing.

scribed before, and which is proposed as a new species of *Tricholoma*.

Tricholoma pseudosordidum sp. nov.

Pileo purpureo-violascente, haud hygrophano, tenui, levi, glabro, 24 mm. lato; lamellis concoloribus, confertis, adnexis et subrotundatis 3.7–5.5 × 3–4.8 μ, inamyloideis, hyalinis, admodum asymmetricis, cruciformibus; cystidiis nullis; basidiis granulis carminophilis destitutis; stipite concolori, innate subfibrilloso, excentrico, deorsum incrassato, 30 × 5 mm.; carne subconcolori, ex hyphis fibuligeris consistente; odore saporeque haud notabilibus. Inter folia in dumeto tropico in Florida.

Pileus "litho purple" or "Saccardo's violet," slightly more sordid in the middle, non-hygrophanous, smooth, glabrous, thin, subumbonate-flat, about 24 mm. broad—Lamellae concolorous but appearing somewhat paler because of a hyaline pruinosity (spores ?), rather broad, subventricose, close to crowded, narrowly adnexed and somewhat rounded.—Stipe concolorous, with faint, hyaline, innate fibrils, and therefore appearing lighter colored than the pileus, eccentric, or irregularly compressed, tapering upwards, about 30 × 5 mm.—Context subconcolorous, non-hygrophanous; odor and taste not distinctive.

Microscopical characters: Spores 3.7–5.5 × 3–4.8 μ, mostly 4–5 × 3–4 μ, hyaline, non-amyloid, smooth, very asymmetric, *viz.* short ellipsoid with a suprahilar depression or applanation and a papilla on the opposite side when seen in profile, but when seen frontally there are two such papillae on both sides of the hilar end which is elongated between them like a projecting cone while the apex of the spores is gradually narrowed to an obtusely rounded tip, the frontal outline thus strongly cross-shaped, the central oil-drop also subangular; basidia 24 × 5.3–6 μ, without carminophilous granulosity, clavate, 4-spored; cystidia none seen; cheilocystidia (mucronate) and pseudoparaphyses (basidiomorphous) scattered at the edge of the lamellae, the size of the basidia, very inconspicuous; trama regular, of thin, somewhat interwoven, hyaline hyphae; epicutis of very interwoven, irregularly filiform, or swollen to clavate hyphae which are mostly repent, only occasionally suberect, thin-walled, very dilutely violet-blue inside (in NH_4OH), subcutis of thicker-walled (but moderately so) hyaline hyphae; all hyphae with clamp connections.

Habitat: In tropical hammock, among fallen leaves of *Ficus, Nectandra,* etc. on the ground, in the Coastal Hammock region near Miami, Dade Co., Florida. The type has been collected by R. Singer (*F 900*) in the Matheson Hammock, and is preserved at the Farlow Herbarium. It fruits in September.

Other species of this section have been described as *Tricholoma goniospermum* Bres., *Tricholoma Cossonianum* R. Maire, and *Tricholoma porphyrophyllum* Imai. Like some of these, *T. pseudosordidum* is similar to *Lepista sordida* in fresh condition. It will be taken for this latter species by the unsuspecting collector.

VI. A "FALSE" CRINIPELLIS

Marasmius Magnoliae Sing. sp. nov.

Pileo intense atrobrunneo, dein ferrugineo-fusco, intersticiis radialibus pallide alautaceo-tinctis, disco maturo crinito-ursino, margine maturo sulcato-rimoso, sicco, margine juniore fimbriato-ciliato, disco 1.5 mm. lato, toto pileo usque ad 5.5 mm. lato, semiglobato, dein plano-convexo, centro subumbilicato, demum subapplanato centro subdepresso frequenter papillato in depressione; crinibus e catenulis hypharum vesiculosarum vel clavatarum, parte libera echinatarum, castanearum, crasso-tunicatarum consistente; lamellis albis, subliberis, distantibus, aequalibus, moderate latis; sporis 8.7–9.3 × 3.5–4.3 μ, hyalinis, non-amyloideis, levibus, ellipsoideo-fusoideis; cystidiis nullis; cheilocystidiis fusoideis, acutis, hyalinis, levibus, integris; stipite atrobrunneo, opaco vel rarius subnitido, ad apicem subattenuato, macroscopice subglabro, insiticio, flexuoso, 10–40 × 0.2–0.5 mm.; carne albida, exigua, ex hyphis tenuitunicatis, inamyloideis, fibuligeris consistente; odore nullo. In petiolis foliorum delapsorum *Magnoliae grandiflorae*, Gainesville, Fla.

Pileus deep brown, then "amber brown" with the depressions of the radiately sulcate-rimose margin pale buff, eventually somewhat pallescent and the margin as a whole about "clay color," hairy ursinous when mature, eventually somewhat glabrescent, the non-sulcate disk about 0.5 mm. broad, the extreme margin fimbriate-ciliate at first, hemispheric then convex, flattened at last and becoming subumbilicate, finally with a slight depression in the center in the middle of which there may be a small papilla, up to 5.5 mm. broad.—Lamellae white, subfree, distant, entire and equal, moderately broad (1 mm.).—Stipe blackish brown, macroscopically subglabrous but at least partially subfibrillose when seen under a lens, opaque, rarely slightly shining, insititious, more or less flexuous, slightly tapering at the apex, 10–40 × 0.2–0.5 mm.—Context white, whitish, very thin, inodorous.

Microscopical characters: Spores 8.7–9.3 × 3.5–4.3 μ, mostly 8.8–9 × 4–4.2 μ, hyaline, smooth, ellipsoid-fusoid, thin-walled, non-amyloid; basidia 26 × 6 μ; cystidia none seen; cheilocystidia about 4–7 μ thick, fusoid, acute, hyaline, smooth, entire; hairs of the pileus consisting of chains of short, vesiculose hyphae which are beset with brown, subpyramidal or cylindric spines of 2.5 μ length; among these hairs there are half-attached epicuticular hyphae which have the shape of ascendant claviculae, arising from each

other's lower side, or forming a chain of normal filamentous hyphae, rarely the hairs consisting of smooth members of free, erect hyphae-chains; these individual hyphae in all cases cited about 11–40 × 7–17 μ, the shortest ones *e.g.* 27 × 23 μ, all thick-walled; hyphae of the context hyaline, non-amyloid, filamentous, with clamp connections.

Habitat: On the petioles of fallen leaves of *Magnolia grandiflora,* very rarely on other parts of the tree, very gregarious in bay heads, fruiting only in May.

This is an interesting species and quite common in North Florida. The type has been collected at Gainesville by the writer and is preserved at the Farlow Herbarium. It has probably been overlooked by other collectors because of its small size, dark color, and unusually limited time of fruting, also because it is often covered by newly fallen leaves of neighboring trees. Macroscopically, it strongly suggests *Crinipellis.* Only the anatomical analysis reveals its affinity with *Marasmius.* In this latter genus, it belongs to the section *Hygrometrici* Kühner, a section in which we have indicated several species with a distinct specialization in regard to their host (*M. rotalis* Berk. = *M. hygrometricus; M. Buxi* Quél., *M. capillipes* Sacc., *M. Hudsonii* (Pers.) Fr., *M. aciculaeformis* B. & C., and others).

VII. THE TROPICAL SPECIES OF OUDEMANSIELLA

In *Oudemansiella,* two species are restricted to the tropics and subtropics. One of them has been named and renamed 30 times, the other not at all.

OUDEMANSIELLA CANARII (Jungh.) Hoehnel, Sitz.-ber. Akad. Wiss. Wien **118**: 276. 1909.
Agaricus Canarii Jungh. Batav. Genootsch. kunst. wetensch. Batav. Verhandl. **17**: [82]. 1838.
Amanitopsis Canarii Sacc. Syll. Fung. **5**: 27. 1887.
Agaricus alphitophyllus Berk. & Curt. Proc. Am. Acad. **4**: 112. 1860.
Mycena alphitophylla Sacc. l.c., p. 305.
Collybia alphitophylla Ito & Imai, Trans. Sapp. Nat. Hist. Soc. **16**: 15. 1939.
Agaricus leucoconis Berk. & Curt. l.c., p. 113.
Mycena leucoconis Sacc. l.c., p. 273.
Agaricus rhodoconis Berk. & Curt. l.c., p. 113.

Agaricus apalosarcus Berk. & Br. Jour. Linn. Soc. Bot. **11**: 520. 1871.
Collybia hapalosarca Sacc. l.c., p. 230.
Agaricus euphyllus Berk. & Br. l.c., p. 520.
Collybia euphylla Sacc. l.c., p. 229.
Agaricus magisterium Berk. & Br. l.c., p. 520.
Collybia Magisterium Sacc. l.c., p. 230.
Agaricus cubensis, Berk. & Curt. Jour. Linn. Soc. Bot. **10**: 282. 1869.
Amanitopsis cubensis Sacc. l.c., p. 25.
Agaricus cheimonophyllus Berk. & Curt. l.c., p. 284.
Armillaria cheimonophylla Sacc. l.c., p. 86.
Agaricus platensis Speg. Ann. Soc. Cient. Arg. **9**: 161. 1880.
Oudemansia platensis Speg. Ann. Soc. Cient. Arg. **10**: 280. 1880.
Oudemansiella platensis Speg. Ann. Soc. Cient. Arg. **12**: 24. 1881.
Oudemansiella apalosarca Hoehnel, Sitz.-ber. Akad. Wiss. Wien **117**: 1003. 1908.
Oudemansiella cheimonophylla Hoehn. Sitz.-ber. Akad. Wiss. Wien **119**: 885. 1910.
Mucidula cheimonophylla Pat. Bull. Soc. Myc. Fr. **15**: 192. 1899.
Mucidula alphitophylla Pat. Bull. Soc. Myc. Fr. **25**: 9. 1911.
Chamaemyces alphitophylla Murr. Mycologia **3**: 91. 1911.
Armillaria alphitophylla Murr. N. Am. Flora **10**: 39. 1914.
Phaeolimacium bulbosum Henn. Monsunia p. 14. 1899.
Pluteus macrosporus Henn. l.c., p. 57.

Pileus initially brownish or rarely hyaline, then very pale grayish brown to hyaline, covered with sordid elastic patches which recall the volva fragments of *Amanita,* these patches sometimes beset with scattered white flocculae, especially at the margin where they form an appendiculate veil, cuticle hygrophanous and at the same time glutinous, but eventually becoming dry, usually transparently striate (one tenth to three quarters of the radius), convex, eventually flattened, 12–92 mm. broad.—Lamellae white, thick at the ground (about 1 mm.) when mature, initially appearing foldlike because of a membranaceous or glutinous, sometimes collariately separating covering, ventricose and broad (3–13 mm.) when mature, tridymous, broadly adnexed or sinuate, the edges sometimes splitting longitudinally; spore print pure white, copious.—Stipe grayish hyaline to white, with a pure white pubescent or flocculose covering above the annulus, flocculose-squamulose below it (or where it should be); dry, solid, bulbous below, tapering up-

wards or downwards, or subequal above the bulb, the latter tapering into an indistinct fleshy-fibrose proliferation within the substratum, 10–65 × 2–11 mm.; annulus obsolete, narrow, or replaced by a more or less belt-like line of floccons around the lower part of the stipe, or sometimes entirely wanting.—Context white, fleshy; odor none; taste mild.

Microscopical characters: Spores 14–23 × 14–19 μ, hyaline, with or without irregular contents, with initially thick (1.5 μ), later thin or thick, smooth and non-amyloid wall and a little projecting asymmetrically attached hilar appendage, globose, without germinative pore; basidia 67–88 × 15.5–22 μ, clavate or slightly attenuate in the upper fifth, constantly with 4 broad-based sterigmata; cystidia sparse to very numerous, ventricose in the middle or below, thin-walled or with thickened walls in the broadest part, always broadly rounded above, sometimes capitate, hyaline, smooth and entire, with or without large, longitudinally elongate vacuoles, 75–180 × 19–30 μ; trama non-amyloid, of somewhat irregularly arranged hyphae but generally regular (not bilateral); marginal (partial) veil made up of subparallel, thin filamentous, hyaline hyphae, 1.5–7 μ in diameter; floccons on the patches of the pileus showing a similar texture; the sordid patches of the pileus themselves consisting of partly melleous to olive brown pigmented tissue of mixed spherocyst-chains and filamentous hyphae (reminding one of the heteromerous structure of some Asterogastraceae, the Russulaceae, and the cuticle of *Smithiomyces mexicanus* (Murr.) Sing.); cuticle pseudoparenchymatous; all hyphae without clamp connections.

Chemical characters: **Methylparamidophenol** on margin of pileus, on lamellae, and on context becoming "dull Indian purple," then "dull lavender," less distinctly positive on the rest of the carpophore.—**Phenol** on context weakly but distinctly chocolate.—**$FeSO_4$** negative.

Habitat: On living trunks, in old wounds, from 0–6 feet and even higher above the ground, also on old roots, and on freshly fallen trunks which are not thoroughly decayed, thus far observed on *Bursera simaruba, Canarium commune, Coccolobis laurifolia, Carya megacarpa, Ficus aurea* and *F. elastica, Liquidambar styraciflua, Nectandra coriacea, Quercus virginiana,* and on various grape vines[3] fruiting from May until October in Florida, and in accordance with the climate, practically around the year.

[3] This host list is based on observations by the writer in Florida (as is the whole description) with the single exception of *Canarium commune* which is given by Junghuhn. This list obviously needs additional data from other areas.

Distribution: Pantropical, in this hemisphere from Florida south to Argentina.

Oudemansiella echinosperma Sing. sp. nov.

Pileo crenato-sulcato, fuscidulo-fuligineo, glabro; lamellis albis, subconfertis, latis, sinuosis; sporis 17–22 × 16–20 μ, echinatis; basidiis cystidiisque giganteis; stipite fuscidulo, subaequali vel bulbum basalem versus incrassato, pseudorhiza praedito; carne alba; magnitudine carpophorarum formas varias *Oudemansiellae radicatae* in mentem revocante. Sao Leopoldo, Rio Grande do Sul, Brazil.

This species is macroscopically very similar to *O. radicata;* the short macroscopical description deriving from notes and from dried material offers little evidence as for its separability from that species. However, the microscopical characters most certainly prove it to be an autonomous species. The spores are 17–22 × 16–20 μ, hyaline, non-amyloid, globose, and *beset by about 38–42 subcylindric to subpyramidal, hyaline spines,* projecting 1.8–3.2 μ and of about the same diameter at their base; basidia 43 × 16.5 μ (sterigmata not seen); cystidia fusoid to subcylindric, subcapitate, often with a resinous incrustation on top, the apex rounded, the walls thin, 100–160 × 9.5–30 μ, mostly 128–130 × 28–30 μ; epicutis formed by 15–32 μ thick globose cells with fuscous plaques of pigment, forming a continuous hymenial layer, connected with a stipe-like chain of cylindric erect or suberect hyphae, rooting in the context of the pileus and forming a sort of a subcutis; clamps not seen.

The type is based on a collection by J. Rick, deposited at the Farlow Herbarium under the name of *Collybia napipes,* and commented on in Broteria **6**: 72. 1907. When Hoehnel studied this specimen and compared it with the Kew types of *Collybia napipes,* he found out that the latter was a different species. He thought, however, as can be seen in notes in his herbarium, that Rick's plant was not different from *O. radicata.* This opinion can not be maintained in view of the echinate spores. Among the Marasmoideae, we now know echinate or stellate spores in two genera (*Marasmius nigripes,* the only *Marasmius* with stellate spores—*Marasmius cyatheae* being not a *Marasmius* but a *Mycenella,* most of which have echinate-warty spores); Marasmioid representatives of other white spored groups with echinate spores are *Laccaria. Lyophyllum tylicolor* (Fr. sensu Lange), and *Fayodia bisphaerigena* (Lange) Kühner. We are now adding *Oudemansiella echinosperma.*

VOLVARIA BOMBYCINA

ALEXANDER H. SMITH

(WITH 1 FIGURE)

VOLVARIA BOMBYCINA (Fries) Quél. Champ. Jura Vosg. p. 114 (80). 1872.

Agaricus bombycinus Fries, Syst. Myc. **1**: 277. 1821.

Volvariopsis bombycina Murrill, Mycologia **3**: 281. 1911.

Pileus (5) 7–12 (20) cm. broad, more or less egg-shaped in the button stages, expanding to campanulate or convex, broadly convex to nearly plane at times in age, surface dry and silky fibrillose or in age becoming somewhat squamulose, rather coarsely fibrillose toward the more or less fimbriate margin, white, finally discoloring slightly to yellowish on the disc, when dried more or less "cinnamon buff" at least on the disc; flesh rather thin, white, soft, mild, odor not distinctive; lamellae free and usually remote, broad and ventricose, crowded, white becoming flesh color from the spores, edges eroded; stipe 6–12 (20) cm. long, 1–2 cm. thick at the apex, solid, somewhat bulbous or merely tapering upward, often curved, white and glabrous, base inserted in the cup formed by the volva; volva thick, membranous, with the upper margin lobed or ragged, surface usually areolate from the checking of the outer layer, whitish at first but soon discoloring to sordid yellowish or near "Isabella color" on the areolae, merely sordid brownish when dried.

Spores pinkish in deposits, 7–8.4 × 5–6 μ, ovoid to ellipsoid, smooth, the walls slightly thickened; basidia four-spored, 28–34 × 7–9 μ, clavate but broadest about 6–10 μ below the apex, projecting 5–10 μ when sporulating; pleurocystidia fairly abundant, variable in size, 34–62 × (9) 12–18 (20) μ, thin walled, smooth, colorless in KOH, more or less fusoid ventricose, the apex drawn out to a subacute proliferation in some, in others merely obtuse; cheilocystidia very abundant, 38–56 (64) × 12–20 μ, ventricose or somewhat ellipsoid, the apex usually drawn out to an abrupt hair-like projection 10–18 × 4–5 μ; gill trama apparently inverse but not reviving well; pileus trama floccose, the fibrils on the surface more or less radially arranged, no clamp connections seen.

Volvaria bombycina.

Usually solitary on decaying wood. Sometimes projecting from knotholes in living trees of maple, beech, elm, etc. It is widely distributed throughout central and eastern United States.

The accompanying photograph was taken by Mr. W. R. Fisher, photographer for the Department of Plant Pathology, Cornell University, Ithaca, N. Y. The specimens were collected in the vicinity of Ithaca by Mr. S. H. Burnham, June 30, 1942, and a portion of the collection and the photographs were sent to me for examination by Prof. H. M. Fitzpatrick. The microscopic characters given in the foregoing description were taken from this collection. In all the material of this species which I have examined the spores measured 6.5–8 × 5–6 μ. Some authors such as Ricken have given slightly larger spore measurements, 8.5–10 × 5–6 μ.

The fruiting bodies of this fungus are very beautiful and attract attention wherever found. Although it is widely distributed and supposedly not rare, I have never had the pleasure of collecting fresh specimens. Fresh material collected here in Ann Arbor and brought in by other collectors, however, has been examined. The fungus has been found rather frequently in the vicinity of Ithaca, New York (nos. *3096; 5340; 8114; 14,821* and *15,308* in the Atkinson Herbarium and nos. *262; 1996; 31,411; 22,851; 22,902; 31,737* in the Plant Path. Herbarium of Cornell University). An interesting story has been brought to my attention in regard to this species. The year before our entrance into the first world war the members of a family here in Ann Arbor were poisoned, some fatally, as the result of eating caps of a species of Amanita. The next year *Volvaria bombycina* fruited on a maple tree at the home of these people, and the story was circulated that some of the spores of the poisonous fungus, which caused the deaths the year before, had escaped from the house, lodged in the tree, germinated, grew and were now producing fruiting bodies. Consequently the carpophores of the *Volvaria* were held in great awe by the neighbors, and soon came to be referred to as the "ghost mushroom." No one, of course, would even consider eating them. This incident appears to me to be worth relating because it illustrates very well how the accidental occurrence of

a fungus may give rise to a superstition, and such a superstition once established is very difficult to dispel. *V. bombycina,* of course, is an edible fungus easily distinguished from the species of Amanita.

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FLAGELLAR STUDIES ON ZOOSPORES OF SOME MEMBERS OF THE MYCETOZOA, PLASMODIOPHORALES, AND CHYTRIDIALES [1]

BERNARD R. ELLISON

(WITH 4 FIGURES)

INTRODUCTION

Since the discovery by Vlk (**19, 20**) that there is more than one type of flagellar structure, mycologists have been increasingly interested in the possibility that the type, position, number, etc., of the flagella might provide a valuable aid in classification and determination of phylogenetic relationships (**3, 22**). This idea is supported by the fact that there has been found to be a definite correlation between these flagellar characteristics and certain physiological and structural characteristics. This is true for both the fungi and the algae. It was with the hope of providing some information that could be used as an aid in determining the position and phylogeny of certain organisms of more or less uncertain relationships that these studies were undertaken.

Vlk's work disclosed that there are three different types of flagella. These are the ordinary, blunt-ended type of flagellum, the whiplash flagellum and the ciliated or "tinsel" type of flagellum. To these may be added a possible fourth type of flagellum, the knobbed flagellum. The occurrence and significance of the knobbed flagellum will be discussed in detail farther on in this paper.

Certain botanists tend to use the terms flagellum and cilium interchangeably. It is the author's opinion that this should be avoided in view of the fact that the term cilium, when used in

[1] This is a condensation of a portion of the thesis presented by the author in partial fulfilment of the requirements for the degree of Master of Science at Michigan State College.

reference to the swimming organelle of a unicellular organism, is well entrenched in zoological literature as referring to those distinctive structures (morphologically quite different from flagella) found on members of the Subphylum Ciliophora, Phylum Protozoa. A flagellum, as contrasted to a cilium, is that relatively long, whip-like, swimming organelle, characterized by having an outer sheath surrounding an inner core. The inner core arises typically from a blepharoplast which is in turn connected to the nucleus by a rhizoplast. It is in this sense that the terms flagellum and cilium are used in this paper.

MATERIALS AND METHODS

Planocytes were obtained both from material collected by myself and from material supplied by other collectors. It would be out of place in this paper to go into detail on the generalities of inducing the production and liberation of the planocytes in various organisms as there are a number of comprehensive articles on this subject (**10**, **11**, **13**, **18**, **21**). In the author's experience, however, there are no generalized methods which are effective in inducing production of the swarm spores. Each species, indeed each specimen, must be treated as an individual and zoospore production must be induced by a laborious process of trial and error. Robert Hagelstein in a letter to the author expressed the opinion that germination studies based on individual specimens do not establish the germination requirements for a whole species by any means. After completing these studies the author found himself in hearty accord with this opinion.

Germination was carried on in hanging drop cultures in Van Tieghem cells or more effectively in culture slides. The latter method was found to the most satisfactory in a number of cases for the reasons that when germination takes a period of several days, condensation on the coverglass, in a hanging drop culture, will allow the drop containing the spores to spread and sometimes be lost by running down the side of the cell. Furthermore, in cases where there seems to be a mass action effect in the germination, the culture slide allows one to use a greater number of spores than is possible or practical in a hanging drop. Temperature was controlled during germination by the use of incubators

and refrigerators when necessary but germination was often carried on at room temperature. Specific methods of obtaining germination for each organism will be discussed farther on in the paper.

Swarm cells to be stained for flagellar structures were collected by means of a micro pipette and placed on a very clean slide. These swarm cells were killed and fixed by inverting the slide over the fumes of a two percent solution of osmic acid. The smear was allowed to dry for several hours and then stained by the Löffler method as modified by Couch (**6**, **7**). The time of mordanting and staining was modified according to trial and error to obtain the best results. In general it was found that mordanting for a maximum time and staining for a minimum time resulted in a sharper staining with less precipitation of the stain on the slide. Having the slides meticulously clean will also help prevent the stain's precipitating. After staining, the preparations were left in a desiccator over night and then mounted in Canadian balsam with a number one cover slip.

Cytological stains were made in cases in which it was necessary to determine whether biflagellate swarm spores were abnormal or were in a stage of division and also to study the neuromotor apparatus. These preparations were stained by an adaptation of the method developed by Cotner (**5**). The strength of the stain was varied somewhat. It was found that the zoospores of the Mycetozoa stain more readily than most other zoospores and a weaker stain was more satisfactory. Smears were prepared in the same way as when the preparations were to be stained by the Löffler method. Rather than introducing a drop of stain into the drop of water containing the dead zoospores as Cotner recommends, it was found satisfactory to apply the stain to the dried smear. After staining, the slide was placed in a desiccator for twenty-four hours, cleared with clove oil and mounted in Canadian balsam. Slides to demonstrate whiplash flagella were made using the method recommended by Couch (**6**), although the author had good results with the Löffler stain for demonstrating both whiplash and tinsel flagella.

The slides were studied under high dry and a clearite oil immersion objective and drawings were made with the aid of a camera lucida.

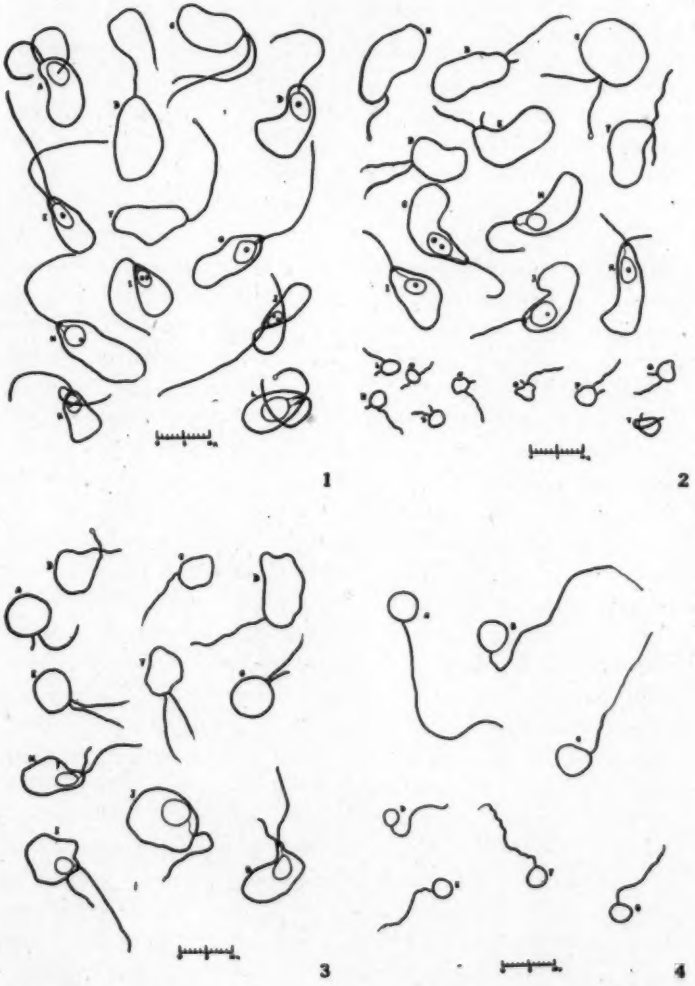

FIG. 1. *Stemonitis ferruginea.* FIG. 2. *Stemonitis fusca,* and *Plasmodiophora Brassicae.* FIG. 3. *Fulligo septica.* FIG. 4. *Nowakowskiella* sp., and *Synchytrium decipiens.*

OBSERVATIONS

Stemonitis ferruginea Ehrenb. The specimen of *Stemonitis ferruginea* used was obtained from Mr. John M. Roberts and was collected by him in Indiana in July 1940. It was stored for approximately two years with no particular attempt to keep the spores viable. When germination of the spores was attempted in January 1942, they were found to germinate readily in distilled water at room temperature. This germination was carried on in hanging drop cultures using garden hose washers in place of the glass Van Tieghem cells. The first germination was observed after forty-eight hours and the spores had germinated up to eighty per cent in fifty-four hours. It is interesting to mention that all attempts to germinate spores from this specimen, after a year's time from the original attempt, failed. Zoospores were collected in a micro pipette and transferred to slides cleaned in hot, soapy, water and stored in ninety-five per cent alcohol. The importance of having the slides perfectly clean cannot be over-emphasized because foreign material will cause the stain to precipitate on the slide. The zoospores were killed by exposure to fumes of two per cent osmic acid for thirty seconds. The slides were allowed to dry in an inverted position. This has the advantage of allowing the zoospores to become better distributed over the slide because it helps prevent them from being attracted to the periphery of the drop. A number of smears were taken for staining. The time of mordanting and staining was varied. Slides selected as being most satisfactory had been treated with the mordanting solution for three quarters of a minute and stained for one and one half minutes. Cytological stains were made by treating a dried smear for five minutes with a five tenth per cent aqueous solution of crystal violet. The stain was washed off with distilled water and the slide allowed to dry for forty-eight hours in a desiccator. The slides were then cleared with clove oil and mounted in Canadian balsam.

Three types or modifications of the flagella were discernable on the slides stained with the Löffler stain. They were as follows: approximately 56 per cent were of the ordinary blunt-ended type; 44 per cent had the knobbed type of flagellum. Biflagellate zoo-

spores occurred in the ratio of fifteen biflagellate to seven hundred and sixty uniflagellate or 1.9 per cent. Of these biflagellate zoospores the flagellate were of various types as follows: both flagella stubbed; one stubbed and one whiplash; one stubbed and one knobbed. Combinations that were not found were those having two whiplash flagella or a whiplash plus a knobbed flagellum. No tinsel type of flagella were found.

The cytological stain showed a large, dark-staining nucleus present in all cells. It was invariably located in the anterior or flagellar portion of the cell. A darker staining endosome is usually present and in some cases two are present. The number of endosomes in the nucleus seemed to have no correlation with the number of flagella. In no case was there more than one nucleus present. Neither was there any indication that the nucleus was preparing to divide or was in any stage of division.

There was no apparent difference in the neuromotor apparatus of the uniflagellate and biflagellate cells. Even in the uniflagellate zoospores the neuromotor apparatus was clearly of a potentially dual nature. This supports the investigations of the Japanese mycologists Sinoto and Yuasa who contend that the zoospores of the Mycetozoa, whether uniflagellate or biflagellate, have two blepharoplasts (**17**, **23**). Two blepharoplasts are present each with a rhizoplast connecting it to the nucleus. Each blepharoplast gave rise to a flagellum in the biflagellate zoospores. There was no apparent difference in the blepharoplasts whether they produced a flagellum or not.

The zoospores were in various stages of becoming amoeboid. Practically all of the zoospores showed the formation of pseudopodia while still in the actively swimming or flagellated state. Other zoospores were seen that had completely lost their flagella and were carrying on locomotion exclusively by pseudopodia. In these cases the entire neuromotor apparatus had disappeared.

Stemonitis fusca (Roth) Rost.: The specimens of *S. fusca* used in the flagellar studies were collected on the Michigan State College Campus in East Lansing. They were collected in April and germination studies were carried on immediately after collection, in hanging drop cultures. It was found that the spores germinated readily in either sterile river water or distilled water at

twenty-eight degrees centigrade. Temperatures either lower or higher did not give as good results. Approximately seventy per cent of the spores germinated under these conditions within ninety-six hours. Little or no germination was obtained at room temperature.

Flagellar and cytological stains were made as for *S. ferruginea*. The relative time of mordanting and staining which gave most satisfactory slides was found to be the same as for *S. ferruginea*.

On those slides stained by the Löffler stain three modifications of the flagella were observed. They were as follows: approximately 30.4 per cent were of the plain type; 14.8 per cent were of the whiplash variety, and 46 per cent were of the knobbed variety. Biflagellate zoospores occurred as about 8 per cent of the total. In the biflagellate zoospores the flagella occurred in the following combinations: plain plus plain; plain plus whiplash; plain plus knobbed; whiplash plus knobbed; whiplash plus whiplash. None were seen in which both flagella were of the knobbed variety. In no case was a flagellum present of the tinsel type. The relative length of the flagellum seemed to have no correlation with the type.

Zoospores of *S. fusca* were the best studied as far as the knobbed flagellum was concerned. These zoospores exhibited the condition in a greater number of cases than zoospores of any other genus or species. It is difficult to say what this modification represents. Knobbed flagella have been observed on members of the Chytridiales and interpreted variously as abnormal forms due to degeneration and old age or due to immaturity (**1**, **8**, **12**). None of these explanations can be regarded as applicable in this case. The percentage of zoospores having the knobbed flagellum was seen to be as great in young, newly germinated cultures as in old. The percentage remained approximately the same in cultures germinated in both distilled water and in river water. The temperature during germination did not affect the percentage. Spores from other specimens were used to make sure that the condition was not due to a peculiarity of an individual specimen. The knobs on the flagella were observed by Dr. Bessey and myself on living zoospores thus removing the possibility that the feature was due to the treatment the zoospores received during the killing and staining processes.

Without additional investigation it would be difficult to make an authoritative explanation of this type of flagellum. Many authors regard the flagellum and the pseudopodium as homologous. If this is true then the sheath of the flagellum would represent the ectoplasm of the pseudopodium and the central cylinder the more fluid endoplasm. If the cylinder, or endoplasm, were for some reason more fluid than is usually the case, a slight extension, instead of remaining as a whiplash, would tend to collect into a drop or knob at the end of the flagellum due to surface tension. This idea is supported in part by the fact that those species having the knobbed flagellum on some of their zoospores have whiplashes on other zoospores. Thus the knobbed flagellum, as seen during these studies, may represent a modification of the whiplash type and not a third and distinct type of flagellum. It must not be regarded in all cases as being an abnormality due to age, environment, etc. It would be interesting to investigate this problem further and determine whether the knobbed condition of the flagellum is characteristic of any particular groups, particularly the Mycetozoa.

In *S. fusca* the dual nature of the neuromotor apparatus is not as evident as it is in *S. ferruginea*. All zoospores remain potentially biflagellate in that they have the usual two blepharoplasts. However, only one of the blepharoplasts has a rhizoplast connecting it to the nucleus. Exactly the same condition is found in the uniflagellate zoospores except that in the former case both blepharoplasts of course produce a flagellum.

A large nucleus is invariably located in the anterior portion of the cell. This nucleus has a darker staining endosome present and occasionally two. There is no apparent correlation between the number of endosomes in the nucleus and the number of flagella on the zoospore. In no case was there anything to indicate that the biflagellate zoospores were in a state of division.

Pseudopodia were present on most zoospores and the zoospores were seen in all stages of becoming amoeboid. In those in which the flagella had been completely absorbed the neuromotor apparatus had likewise degenerated.

Fuligo septica (Linn.) Gmel.: Specimens used in the study were collected shortly after the formation of the sporangium. These

germinated in 96 hours in both distilled water and in sterile river water at room temperature and also at 28 degrees centigrade. The time of germination was cut down to 48 hours at room temperature by wetting and drying the spores. This was the only one of the many slime molds worked with which was induced to germinate in less time by the wetting and drying method as recommended by Jahn (**13**).

Three modifications of the flagellar structure were found in the following proportions: 52 per cent of the whiplash type; 47 per cent of the blunt ended type; and less than one per cent of the knobbed type. Since these are probably all modifications of the whiplash type it might be more accurate to say that all were of the whiplash type. None of the zoospores had flagella of the tinsel type.

Biflagellate zoospores were common, amounting to about 26 per cent of the total. These had the following combinations of flagella: blunt ended plus blunt ended; blunt ended plus whiplash; whiplash plus whiplash; and blunt ended plus a knobbed flagellum. A combination not observed was whiplash plus knobbed. The relative length of the flagella varied from markedly heterokont to isokont.

Like the other members of the Mycetozoa studied, two blepharoplasts were always present in both the uniflagellate and the biflagellate zoospores. In the case of the biflagellate condition each of the blepharoplasts produced a flagellum. Only one of the blepharoplasts had a rhizoplast connecting it to the nucleus, however, in the case of the uniflagellate as well as the biflagellate zoospores. In this regard the zoospores resemble those of *Stemonitis fusca*. In the uniflagellate zoospores the single rhizoplast connects the nucleus and the blepharoplast which produces the flagellum. A rather large but lightly staining nucleus is present in the anterior part of the cell. No endosomes were observed in the nuclei. There was no indication that any of the biflagellate zoospores were in a stage of division. None of the zoospores had flagella of the tinsel type.

Plasmodiophora Brassicae Woronin 1848: The flagellar structure of *Plasmodiophora Brassicae* is of particular interest at the present time due to a tendency on the part of some authors (**14**)

to place the genus in the family Woroninaceae. This is done largely on the basis of Ledingham's research (**15**, **16**) which showed that some genera of the Plasmodiophorales (*Plasmodiophora* and *Spongospora*) had two flagella rather than one as in the original description. It has been shown, however, that the slime molds as a group are potentially biflagellate in that they have two blepharoplasts and often possess two flagella (**9**, **17**, **23**). Consequently the possession of two flagella should not necessarily make it impossible to consider the Plasmodiophorales as being allied to the slime molds. As some other members of the Woroninaceae have been shown to have a tinsel type of flagellum and the slime molds have only the whiplash or modified whiplash type, the presence or absence of the tinsel flagellum on *Plasmodiophora* should be a diagnostic character of some importance and would tend to confirm its inclusion in the Woroninaceae group on the one hand or its alliance to the Mycetozoa group on the other.

Zoospores used in this study were obtained by germinating spores from infected roots. A number of different methods recommended in the literature for obtaining germination of the spores were tried with little success. Chupp (**4**) recommended germinating the spores in a muck soil filtrate (pH not specified) and incubating them at an optimum temperature of 28 degrees centigrade. He found that germination dropped rapidly as the incubation temperature was lowered and he was able to get little or no germination at room temperature. He was unable to obtain infection of cabbage seedlings in the greenhouse during the winter. He was also unable to obtain germination of the spores in distilled water. Wellman (**27**) on the other hand found the optimum temperature for germination to be not over 25 degrees centigrade with the percentage of germination dropping off rapidly as the incubation temperature was raised. He recommended that the spores be germinated in considerable quantity because there seemed to be some mass effect. He reported germination as being good in distilled water. Ledingham, in a letter to the author, reported that he found germination to be satisfactory when the spores were wet and dried a number of times and then incubated at room temperature in tapwater with a pH of 8. He used distilled water also and obtained satisfactory germination. He recommended that

the spores be germinated in considerable quantity because of the mass effect. These ways were tried as recommended and many others including the placing of sterile, excised, root tips in the cultures in hope that the presence of the living host tissue might have a stimulatory effect on the spores. Rain water and melted snow water were tried. The pH was manipulated by adding small amounts of lactic, acetic, and hydrochloric acid to the various media. Minute amounts of hydrogen peroxide were added to the media in an attempt to supply oxygen to the spores. Oxygen and air were bubbled through the cultures in an attempt to induce germination. Results were either entirely negative or the germination was so very slight that it was impossible to obtain the zoospores. A spore was once observed germinating, by Dr. Bessey and myself, which had become attached to one of the root hairs of an excised root placed in the culture. The spore case was seen to be split and a small amount of protoplasm had oozed out. Attached to this bit of protoplasm two actively beating flagella could be detected. Unfortunately this zoospore was lost in attempting to transfer the rootlet from the culture dish to a slide for staining. Our observations confirm those of Wellman (21) on the germination of the spore. He reports that flagella are produced almost immediately after the first bit of cytoplasm comes through the break in the spore case and from that time on the partially germinated spore swims about actively. As considerable time may elapse before germination is completed it makes the last stages of this process exceedingly hard to observe. It is not altogether surprising that spores of *P. Brassicae* should vary in their requirements for germination because of the existence of physiological races in this organism.

The best germination obtained was obtained under the following conditions: infected roots which had been frozen and thawed several times over a two month period were macerated in a mortar. Distilled water was added and the mixture stirred up to place the liberated spores in suspension. The coarser material was allowed to settle to the bottom and the supernatant liquid with the spores in suspension was decanted off and strained through cheese cloth. It was then placed in the centrifuge and rotated slowly to throw the coarser plant material to the bottom. The

spore suspension was then poured into another centrifuge tube and centrifuged again, this time more rapidly so that the spores were thrown down. The water containing most of the bacteria was then poured off. Sterile distilled water was added and the spores stirred up into a suspension again. They were centrifuged once again. This washing was repeated a number of times depending on how numerous the bacteria were originally. The spores were eventually left to germinate in a Syracuse watch glass containing sterile distilled water made just acid by adding acetic acid with litmus paper as the indicator. The spores were incubated at twenty-five degrees centrigrade. On the third day a fair degree of germination had taken place.

Zoospores to be studied for flagellar structure were collected, killed, and stained with the Löffler stain and Couch's whiplash stain in the same way used for the slime molds. Those slides selected as most satisfactory of those stained with the Löffler stain were those that were mordanted for forty-five seconds and stained for one minute.

The zoospores were all of the biflagellate type and in most cases markedly heterokont. In some of them the shorter flagellum was so reduced as to be almost unnoticeable. In one zoospore found, the two flagella were very nearly isokont. The flagella were in all cases of the blunt ended type. No tinsels or whiplashes were to be found on any of the flagella.

Nowakowskiella sp. The zoospores were obtained from a culture that had been maintained in the laboratory for about a year. Those zoospores to be studied for flagellar structures were handled and stained with the Löffler stain in the same manner as described for other zoospores. The best results were obtained by treating the slides with mordant for three quarters of a minute and with the stain for half a minute.

Two types of flagella were observed on the slides stained by the Löffler stain and also on the slides stained by the Couch whiplash stain. These were the definite whiplash type and the blunt ended flagellum. The whiplash varied in length from very short to some having the whiplash as long as the flagellum proper. It was found that in the cases where the whiplash was extremely long there was a corresponding reduction in the length of the flagellum proper.

Less than one per cent of the zoospores had two flagella. These flagella were either of the whiplash or blunt ended in all combinations. No tinsel flagella were found. These abnormally biflagellate zoospores were uninucleate and therefore are not comparable to the binucleate zoospores described by Cotner for *Blastocladia* which were due to the failure to complete the cleavage into the normal, uniflagellate, uninucleate cells. What is the cause of this abnormal doubling of the flagella has not been determined.

Synchytrium decipiens Farlow: The specimen used was collected by Dr. Bessey on a leaf of *Amphiocarpa dioica*. The zoosporangia were scraped from the sori on the leaf and transferred to a drop of distilled water in a hanging drop culture where they germinated in three hours at room temperature. The zoospores were collected, killed, and stained by the Löffler stain and Couch's whiplash stain in the same manner as mentioned before.

Only uniflagellate zoospores were found to occur. The flagella of these were all of the whiplash or blunt ended type. The whips were relatively short, varying from none at all to about two microns in length. Approximately fifteen per cent had no whiplash at all.

SUMMARY AND CONCLUSIONS

Mycetozoa and *Plasmodiophora:* The work done on this problem has confirmed the contention of Sinoto and Yuasa (**17, 23**) that the swarm cells of the Mycetozoa have two blepharoplasts. It shows that the Mycetozoa may be regarded as potentially biflagellate and that as Gilbert (**9**) and the Japanese authors, mentioned above, pointed out, two flagella are frequently found on the swarm cells. The writer definitely demonstrated their presence in *Stemonitis ferruginea, S. fusca* and *Fuligo septica*. It establishes the type of flagellum for these forms and by inference the entire Mycetozoa as being the whiplash type or a modification of that type, *i.e.* blunt ended or knobbed.

A germinating sport of *Plasmodiophora Brassicae* was seen to have two actively beating flagella, thus confirming Ledingham's investigation that indicated that the zoospores of *P. Brassicae* were biflagellate. The investigations of the flagellar types of this swarm cell lend support to the view that their flagellation is of the My-

cetozoa type. Certain authors wish to place the members of the Plasmodiophorales in the family Woroninaceae. Some members of this family, however, have been shown to have the tinsel type for one flagellum and the whiplash type for the other. In view of this, *P. Brassicae*, the type genus and species of the Family Plasmodiophoraceae and the order Plasmodiophorales, must be excluded from relationship to the Woroninaceae due to its type of flagellation.

Synchytrium decipiens and *Nowakowskiella* sp.: This investigation shows the flagellar type of these two genera to be of the whiplash type. This lends support to the assumption that the flagellar type for the Chytridiales (in the narrow sense of the term) is of the whiplash or modified whiplash type.

Certain authors homologize the flagellum and the pseudopodium. It is the author's opinion that on the basis of this hypothesis one is able to homologize the blunt ended, knobbed, and whiplash flagellum. The knobbed flagellum, as described in the literature prior to this paper, is described as an abnormal and degenerate condition due to age etc. This is clearly not the case in the organisms reported in this paper as having the knobbed type of flagellum. The indication is very strong that the knobbed flagellum is a modification of the whiplash type and not always degenerative in its nature.

This research was carried on under the supervision of Dr. Bessey and the author would like to express his thanks and appreciation to Dr. Bessey for suggesting the problem and for the aid and inspiration given during the entire course of the research, also for his help in the preparation of this article.

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FIG. 1. *Stemonitis ferruginea* Ehrenb. A, B, C, and F stained with the Löffler stain, D, G, H, I, J, K, and L stained with a crystal violet cytological stain. B and C show the whiplash type of flagellum. F and L the knobbed flagellum.

FIG. 2. A to K, *Stemonitis fusca* (Roth) Rost. A to F stained with the Löffler stain, G to K with the crystal violet cytological stain. A, G, and H show the knobbed flagellum, B and D the whiplash flagellum. L to T, *Plasmodiophora Brassicae* Woronin. All are stained with the Löffler stain and show the blunt ended type of flagellum.

FIG. 3. *Fuligo septica* (Linn.) Gmel. A to G stained with the Löffler stain, H to K with the crystal violet cytological stain. B shows the knobbed type of flagellum, C, E, F, and G show the whiplash type.

FIG. 4. A, B, and C, *Nowakowskiella* sp. All stained with the Löffler stain. B and C show the whiplash type of flagellum. Note the extreme development of the whiplash in C. D to G, *Synchytrium decipiens* Farlow. All stained with the Löffler stain. E, F, and G show the whiplash type of flagellum.

MYCOLOGY PRESENTS PENICILLIN

CHARLES THOM

A conservative medical official recently told a technical audience that "Penicillin is the most potent agent" * * * "ever encountered, which produces no bad effects upon the patient." He then listed as conspicuous among microbial enemies of man which respond to penicillin, *Gonococcus, Meningococcus, Streptococcus hemolyticus* and *S. viridans, Pneumococcus, Staphylococcus, Clostridium tetanus* and *C. Welchii, Corynebacterium diphtheriae* and *Actinomyces bovis.* Such a list justifies the caption "miracle drug" put upon it by another medical scholar who is rarely swayed by impulse. I am asked to trace the mycological history of the discovery and development of penicillin to its present place among things worth while. Since there are some misunderstandings as to its early history, the data will be presented in as verifiable order as possible.

The first ten years. In 1929, Alexander Fleming reported the discovery and naming of penicillin. The essential facts of this discovery consisted (1) in the observation that a particular mold growing as a contaminant in a culture of *Staphylococcus aureus* inhibited the bacterium, *i.e.* that the *Staphylococcus* could not grow in a zone surrounding the mold colony. This observation was already well known as a phenomenon seen frequently when molds and bacteria are found growing together. Most commonly the observation is reversed—a mold fails to develop normally, if at all, in the presence of large numbers of bacteria. (2) Fleming went further and showed that the mold had produced an inhibiting substance in the nutrient substratum which could be separated from the fungus itself and used to inhibit certain bacteria. He tabulated a series of bacteria susceptible and other bacteria resistant to the inhibiting substance. (3) Since the mold was a species of the great genus *Penicillium,* he called his inhibiting substance penicillin. (4) Not being a mycologist, he undertook to

identify his mold from the literature and selected the name *Penicillium rubrum* Biourge which was published for it in his 1929 paper. (5) He described the use of penicillin in laboratory practice as facilitating the culture of certain gram-negative organisms by inhibiting their most usual gram-positive accompanying species.

Fleming did not pursue the chemical study of penicillin, survey its production by other molds, or by other strains of the same species. In subsequent papers (1931, 1932, 1936) he elaborated his studies upon the same general line with the same strains but did not broaden the field greatly.

The Fleming organism comes to America. On April 29, 1930, Prof. Harold Raistrick of the School of Tropical Medicine in London wrote as follows to Thom in Washington: "The other culture, labelled *P. rubrum* (?), was received from the British National Collection of Type Cultures and bears their catalogue number *3127*. It was originally isolated by Dr. Alexander Fleming of St. Mary's Hospital, London, and is described by Fleming in a paper * * * (see citation). I am carrying out a piece of work on this species and since, as you will see if you refer to the original paper, this diagnosis of it is very doubtful, I wonder if you would be good enough in this case to let me have an authoritative statement as to its identity." A culture of the Fleming organism accompanied the letter. From this letter it is clear that Fleming's culture was in the hands of St. John Brooks before April 1930. Raistrick's laboratory began work with it before May 1, 1930, hence it was available to any worker. Raistrick, from his own knowledge of molds, did not accept the identification of this organism as *P. rubrum* (either according to Stoll or to Biourge). To that letter, I replied (June 30, 1930): "For this other culture, however, I am obliged to you since I was anxious to know what Fleming's organism would be like. I have cultivated it under several different conditions and cannot agree with his nomenclature as *P. rubrum* either in the sense of my 1910 paper or in the sense of Biourge's Monograph. In fact, I believe his culture, although showing some divergences in culture reaction, to be closer to *P. notatum* of p. 264 in my book than to the group discussed on pages 249 to 250 as indicated by the nomenclature used" (by Fleming). The culture received from Raistrick was put in

the Thom collection as *144.5112.1*. The corrected name appears in Raistrick's papers and was accepted by Fleming in his 1932 paper. Nevertheless, requests for *P. rubrum* were frequently received during the several succeeding years. These were usually answered by sending the Fleming culture and a letter explaining the corrected nomenclature.

The importance of corrected nomenclature here lies in the fact that recognition of Fleming's organism as one of the great and universally distributed *Penicillium chrysogenum-notatum* group opened at once, as we saw it, the possibility that research among related organisms would show penicillin production to be common to the group, hence would enable us to choose among available penicillin-producing forms. Raistrick was at that time the outstanding advocate of the specificity theory in biochemical reactions. His hypothesis was that the formation of a particular biochemical product was tantamount to proof that a particular species of mold was present as the producing agent. The product was assumed to be unique to the species; conversely, absence of the special product was proof that the mold did not belong to the species. Hence a chemical analysis could be depended upon to disclose the identity of the mold responsible for the findings. In his dealings with us his analysis had actually caught mistaken identifications often enough to give it presumptive value. Rigorous comparative work with cultures seems to prove that strains morphologically identifiable as members of a related series may be expected to produce reactions of the same kind or nature, but that these individual reactions may differ greatly in quantitative expression. Concretely it has been shown that among penicillin producers, the amount produced by one strain may be 100 times as great as that produced by another, or, under a standardized routine, one may produce abundantly, another give no penicillin. But there is fair reason to believe that under adequate investigation all related forms will produce the substance unless the capacity has been entirely dropped by mutation. The *P. notatum* series *does* mutate conspicuously. Specificity definitely fails when we find what seems to be real penicillin produced by strains of *Aspergillus flavus* and *A. flavipes* representing not only another genus but two quite contrasting groups within that genus. In the case

of penicillin, the English workers long insisted that penicillin is produced only by Fleming's organism and its derivative strains. Finally, however, Raper, Alexander, and Coghill, working in Peoria piled up proofs that strains isolated from soil and other substances from widely separate regions would produce it, and finally that some of them would produce it in considerably greater quantity. Strangely enough the Fleming strain and its derivative or substrains remained the best producers of penicillin for many months after the collection and testing of other members of the *chrysogenum-notatum* group began. In spite of fantastic stories of its single appearance in St. Mary's Hospital Laboratory, one familiar with the inhibition of mold cultures by bacteria is compelled to believe that *P. notatum* was an "old settler" there, which survived by mutation and selection until at last one member of the lot was conspicuously able to "hold its own" against its bacterial competitors.

So much for one strain, the Fleming organism, and its emigration to America. It was distributed from the laboratory in Washington by Thom and Raper to all who asked for it from 1930 onward. How many other transfers from the English type culture collection reached America we do not know but none have come to our attention. There is one reference to Bornstein as obtaining his culture from Fleming. In a file of letters before me, we have the record of its distribution to great university laboratories, hospitals, and to manufacturing chemists who are now producing penicillin.

The next strain that we know about (also a Fleming derivative) was brought to America by Drs. Florey and Heatley and delivered to me personally on July 9, 1941. To ensure a separate record it entered the collection as *144.5767*. It was passed by Heatley to the Northern Regional Laboratory at Peoria and to an unlisted number of manufacturing laboratories, hence derivatives from Heatley's strain may appear anywhere.

This digression from the story is made merely to record the story of the penicillin-producing culture. We return now to the development of our information about penicillin.

As indicated in Raistrick's letter already quoted, his laboratory began to study penicillin in 1930; this work was reported by Clut-

terbuck, Lovell and Raistrick in 1932. They included *P. chrysogenum* Thom and *P. notatum* Westling (type) strains, with Fleming's strain and grew the three molds under their standradized procedure. No penicillin was obtained in their cultures of *P. chrysogenum* and *P. notatum* (Westling's strain); they therefore concluded that penicillin is a product unique to Fleming's organism. They identified and discussed chrysogenin as the yellow pigment produced by *P. chrysogenum*. This pigment is produced so commonly along with penicillin that its presence is often regarded as indicative of a profitable culture. It is also produced without penicillin, hence the correlation is not entirely dependable. While Raistrick's group believed their culture methods and their chemical work had paved the way to the analysis of penicillin, they did not complete the study. The instability of penicillin, together with the very small yield per unit of material used, appears to have led them to close their investigation.

Roger D. Reid obtained his first culture of the Fleming organism from us in November 1930, another transfer in July 1931. His studies published in 1933, 1934, and 1935, covered the conclusion that penicillin is bacteriostatic instead of bacteriolytic, and detailed its reactions to light, gases and temperature, effects of distillation, dialysis; in the main they confirmed and extended the work of Fleming, and of the Raistrick group without going beyond the chemical and bacteriological laboratory aspects and without suggesting possibilities of development to major usefulness. There is a gap in publication concerning penicillin between Fleming's note to the Second International Congress of Microbiology in 1936, and 1940. Survey of laboratory correspondence during the period from 1933 to 1940 shows that requests for Fleming's organism came from a number of the great laboratories engaged in bacteriological research but not from the pharmaceutical manufacturers. Their immediate response to Florey's paper in 1940 suggests that they had not previously obtained the organism from other sources. In this period then whatever work was done in the laboratories using *Penicillium notatum* was not reported except the paper of Bornstein who tested penicillin against "Enterococci and other Streptococci."

Florey in his recent paper (Endeavor **111** (9): 3–14. Jan. 1944) records that his group began to work on penicillin in 1938. This study was reported in the *Lancet* Aug. 24, 1940 by Chain, Florey, Gardner, Jennings, Orr-Ewing, Sanders, and Heatley. Thus Florey seems to have inspired the work done by a considerable group of workers in Oxford University and in collaborating hospitals. For the purpose of the moment, we will disregard the mass of constructive biochemical and bacteriological work done by the group. The pharmacological work detailed in that paper, followed by the 1941 report by Abraham, Chain, Fletcher, Gardner, Heatley, Jennings, and Florey, aroused widespread interest. Turning again to the laboratory file, requests for Fleming's organism for use by manufacturing chemists began in a letter dated Sept. 23, 1940, another Oct. 1, 1940. One university laboratory asked for it August 21! Was there any connection? Within a half year after the publication of the 1941 paper, laboratories and pharmaceutical houses well distributed in the United States and extending to Mexico and Brazil were supplied with transfers of the Fleming organism (*144.5112.1*).

One phase of the work of the Oxford group must be briefly presented. To facilitate comparison of the results of successive cultures and the value of solutions of unknown origin an assay method was devised by Dr. N. G. Heatley.—As described in outline of Florey:

"An agar plate is seeded with the test organism—*Staphylococcus aureus* has been used as a routine—by pouring on a broth culture of the organism, draining off the excess, and drying the plate for 1–2 hours in the 37° C. incubator with the lid raised. Short open-ended cylinders of glass or vitreous porcelain are then placed on the surface of the agar, and the solutions to be assayed are placed in the cylinders. After incubation, the surface of the agar becomes covered with a continuous film of bacterial growth except for a circular zone around each cylinder where the penicillin has diffused out and inhibited growth. The diameter of this zone is related to the concentration of penicillin in the solution in the cylinder, and by setting up solutions containing known amounts of penicillin a curve relating the two can be drawn. The actual diameter of the zone produced by any given solution varies slightly from day to

day, since it depends on a number of factors, some of which are difficult to control in practice; but the variation can be countered by including one or more solutions of known strength in each assay.

"For the same reasons which apply in the case of other biologically active agents of unknown purity, it was found convenient to express the antibacterial activity of penicillin in terms of some standard preparation of penicillin. The 'unit' originally taken for convenience in this laboratory, only, has since been adopted as the 'Oxford unit' by some other workers. It was defined originally as that amount of penicillin contained in 1 ml. of a certain purely arbitrary stock solution. The latter was exhausted long ago, but other primary standards, in the form of dry preparations, were standardized against it. Until penicillin is obtained in a clearly characterizable form the only way in which the potency of a given solid or liquid preparation can be measured in terms of Oxford units is by a direct comparative assay against a preparation containing a known number of these units."

Penicillin—comes to America. While the 1941 paper was still in press, Dr. Florey obtained the support of the Rockefeller Foundation in London (see *R. F. Review* for 1943) and was sent with his associate, Dr. N. G. Heatley, to New York in July 1941. There are discrepancies in the stories told as to what happened in New York. We know from our laboratory records that certain manufacturers already had Fleming's organism and may infer that they were already working with it. Enough that Florey and Heatley did not establish American connections from the Rockefeller offices in New York. The Rockefeller Foundation sent them to the Medical Section of the National Research Council in Washington on July 8, 1941. Since problems concerning *Penicillium* had long been handled in the Department of Agriculture, the project was referred to us directly by telephone. Arrangements were completed by telegraph on July 9th to turn the project over to the Northern Regional Research Laboratory of the U. S. Dept. of Agriculture at Peoria, Illinois, and on July 13th Florey and Heatley were in the Peoria Laboratory where they had the coöperation of a group of men with long experience in mold fermentation, including Herrick, May, Coghill, Ward, Raper, Moyer, and others.

Florey stayed only a few days, then turned to other interests; Heatley continued in Peoria for a time, then visited a number of large manufacturers in the effort to stimulate production. He worked in one plant for several months before returning to England.

Thus the penicillin project reached America and fell into the hands of a great government laboratory from which has come most of the fundamental work that has made large scale production possible.

The problem of production became a study in mold physiology in which our measure of success has directly represented the accuracy of our delimitation of these problems and the adjustment of our procedures to the fundamental principles determined. Some of the problems encountered in developing penicillin must be discussed.

Aerobiosis. *Penicillium notatum* is aerobic. It grows in nature upon the surface of a substratum—floats as a scum or blanket of mycelium upon fluid or forms a velvety green mat on solid or semisolid food. Its hyphae penetrate normally perhaps one or two millimeters but are restricted from going more deeply by lack of air. Naturally, then, the first development of penicillin production was "still" culture—colonies grown in broad bottomed flasks, shallow pans, milk bottles, etc. Effective use of the food material was found in layers less than 25 mm. (1 inch) deep. To handle a large volume of culture substratum in such shallow culture, enormous areas must be grown. This required thousands of bottles or flasks; hence represented a cumbersome process expensive in labor, but produced a favorable yield. Such producing plants were quickly established and yielded most of the penicillin produced in America from 1941 to the end of 1943.

Ventilation. The excess of carbon dioxide produced necessitates the maintenance of a constant flow of air free from other molds and bacteria. The necessary sources of pure air, filters and circulating machinery are usually closely guarded secrets of factory installation.

Pure culture. *Penicillium notatum* is a vigorous grower only under optimum conditions. At best many other molds if present will overgrow it and destroy the product. Penicillin inhibits

gram-positive bacteria but not the commonest gram-negative species. The ubiquitous *Escherichia coli* if it enters as a contaminant will render a culture worthless. Rigorous exclusion of other organisms is essential in handling penicillin production.

Variability. Most strains of *Penicillium notatum* are exceedingly unstable. In miscellaneous culture variants or mutants are very common. To maintain continuous production, standardized culture must be developed to maintain a dependable strain. Without such care, the stock may deteriorate and become worthless for penicillin production.

Selected strains. Systematic selection of variants from the Fleming organism resulted in greatly increasing the yield above that obtained by the Oxford workers but the limit was soon reached and the yield per litre of solution was still fantastically small. Surveys have therefore covered thousands of samples of soil from widely separated regions and moldy substances wherever found. Fleming's organism has been replaced already by better yielding strains and thousands of new strains have been tested in Peoria, Madison, Minneapolis, Cold Spring Harbor, Palo Alto, and elsewhere. Radiation of cultures by many procedures is being tested. No one knows where the limit may fall. In the main the results of radiation merely increase the number of variants handled without greatly increasing the yield beyond that produced by strains met in the survey of natural mold sources.

The culture medium. Penicillium notatum is widely distributed in nature. It appears in culture from soil, especially in rich cultivated soil since it depends on organic remains for growth. It is fairly common in or on miscellaneous food stuffs. In pure culture it will grow upon many types of culture media and usually produce some penicillin. As Florey and Heatley brought their strain to America and grew it on routine media the yield of penicillin was very small. In the hands of the Peoria laboratory it was quickly shown that the addition of a small percentage of the "corn steeping liquor" long used in the yeast industry would multiply the yield many times. Sterilized yeast, brown sugar, wheat bran and cabbage juice have been reported favorable. Thus far "corn steeping liquor" ("corn steep") is preferred by all but the few

manufacturers who have had experience with the bran process and claim good results.

Since the exact component of either of these products responsible for penicillin formation is not known, two hypotheses have been offered to account for the findings (a) that penicillin is an extracellular product due to agents secreted by the mold acting upon the substratum. Hence penicillin, whether produced by *Penicillium notatum, Aspergillus flavus* or *A. flavipes,* is the result of secreted agents, probably enzymes, acting upon specific materials present in the substratum or (b) the alternative hypothesis which assumes intra-cellular biochemical activities which result in the secretion of penicillin and postulates that the same intra-cellular activities must be present in any mold producing penicillin. Proofs are not at present available.

Temperature. The optimum temperature for penicillin production has been found to be 24° C. Lower temperatures unduly slow the activity; higher temperatures set free destructive agents. In rooms with thousands of fermenting units control apparatus must be adequate to absorb excess heat of metabolism.

Penicillin production marks a physiological stage of the colony. Penicillin appears to be produced at nearly the same stage in mold development as a crop of spores (conidia). Grown as a single colony in the center of a petri dish, *P. notatum* forms a circular colony often showing radiating wrinkles like spokes of a wheel. Biourge called the series Radiata because of this appearance. By the time the colony is three or four days old a definite central area green from ripening spores is evident. During a growing period of ten days or more, the colony shows an outer colorless band or zone, passing over to green very quickly. Hence at 10 days old there will be an overripe central area, a zone of active fruiting and a narrow outer zone of colorless hyphae actively growing away from the green center. Sampled by removing disks with the cork borer, the zone of production is found to coincide in general with the zone of fresh sporulation. Penicillin in these old cultures diffuses out into the culture medium and is detected in the assay plate, for two or three centimeters beyond the margin of the colony. The petri dish colony described shows areas old and even disintegrating, areas of maximum penicillin production, and areas of

mycelial growth not yet at the producing stage. Such a unit is inefficient when maximum yield is the aim of culture.

To make a shallow pan or bottle culture become an effective producing unit, therefore, it is desirable to inoculate with spores spread evenly over the surface and spaced closely enough to insure that the mycelia developing will quickly come into contact, intertwine, and to some degree anastomose to form a complete blanket of mycelium over the entire surface simultaneously. In this way the entire mycelium acting as a unit will reach the physiological stage for penicillin production at one time and present the largest possible opportunity for simultaneous effect upon the medium.

In sugar containing media, *P. notatum* produces acidity—glucose is changed to gluconic acid. If the pH is allowed to fall to 4 or 4.5, glucose-oxidase variously called "penatin," penicillin-B, notatin, etc., is produced. This is much more intensely antibiotic than penicillin but it is also toxic. Cultures kept above pH 6 do not show this toxic substance. Properly buffered to maintain the cultures in the developmental period above pH 6, the pH rises with the maturity of the colony to pH 7, 7.5, 8.0, even to 8.5. Penicillin seems to be mainly produced between pH 7.5 and 8.0 to continue development perhaps to pH 8.3. As the culture reaches pH above 8.3 destructive agencies appear and the penicillin breaks down quickly. Much work upon the activity of the penicillinases concerned in the destruction of this product leaves many points unsettled. In practice, the absolute maximum yield is usually sacrificed to allow a margin for safety.

Submerged culture. Molds of this group germinating below the surface of liquid media commonly form abnormal stringy masses of hyphae without fruit. Nevertheless the advantages of tank culture from an industrial viewpoint lead workers to explore the possibility of conducting mold fermentation under submerged condition. Thus *A. flavus* has long been used in this way. Later Herrick, May, Ward, *et al.*, developed the production of gluconic acid in tanks by *Penicillium chrysogenum*. The way was thus prepared to develop a practice for handling the nearly related *P. notatum* in submerged culture.

Mold physiology is fundamentally the same no matter what we want the organism to do. Our task is to adjust our practice to the demands of the mold. First among these demands is air. On a laboratory scale, a flask ¼ to ⅖ full of liquid inoculated with a mold may be aerated by placing it upon a shaking machine, or by diffusing air throughout by any standard practice or by a rapid agitation by some stirring machine. Thorough aeration by either method keeps the liquid in rapid motion. The mold spore or mycelial fragment under these conditions becomes a growth center from which cells as hyphal components radiate in all directions. In response to tension, instead of forming long slender thread-like hyphae the cells shorten, increase in diameter so that the mycelial development is not a mat, a membrane or a stringy mass but approximately a globose mass or pellet. The same picture is produced in the shaken tube or flask, or the blown vat containing thousands of gallons of liquid. If the agitation is stopped for a few moments the mass of pellets often occupies ⅔ or more of the volume.

The difficulties of tank production include the necessity of a constant supply of sterile air carried to every drop of the liquid or every pellet of mold, maintenance of the correct temperature against a heightened rate of metabolism, buffering to control the reaction, together with the development of testing methods which will determine the exact physiological condition in the tank of liquid at any time. In other words, the percentage of efficiency of a factory procedure is measured by the perfection of adjustment between that practice and the physiological requirements of the particular strain of mold in use. Readjustments may be expected to be needed every time an untried strain of mold is substituted for one previously successfully handled.

In spite of many losses and delays tank installations were gradually developed and put in operation. By January 1944, production began to be possible on a large scale. Early in the spring of 1944 totals of production per month had reached somewhere in the 100 to 200 billions of Oxford units per month, release of penicillin for civilian use in hospitals followed early in May and marked the end of the developmental period.

In addition to various types of surface and tank culture, the great interest aroused by the publication of Florey's papers and various popular reports led many laboratories to begin work upon the problem, each following lines based upon the background or the imagination of the workers. Some of the proposals had no constructive value—some have made definite contributions. Clifton (*Science* **98** (2533): 21. 1942) in California proposed the use of a type of generator patterned after that used in the vinegar fermentation. In this a trickling stream of culture medium passes slowly through a mass of shavings covered with mold thus constantly replacing the liquid in contact with the mycelium. Clifton did not report work upon an industrial scale. Others have shown that mycelium of *P. notatum* fed by a continuous flow of fresh culture medium can be made to continue producing penicillin for about three times its period of activity shown by surface or "still" culture and a dozen times its effective life in tanks. Whether it is adaptable to an industrial procedure remains unsettled.

Other workers reading Florey's group reports noted that the crude filtrate from cultures of the Fleming organism freed from mold protein and other pyrogens had been used intravenously in England. The raw filtrate had been used directly for external treatment. Both uses were without ill effect. Such materials could be readily prepared in the culture laboratory. While it was recognized that this cruder form of penicillin deteriorated rapidly at room temperatures, it was also known that it could be kept for considerable periods in the refrigerator. It was thus possible for the hospital laboratory to maintain penicillin solutions sufficiently strong to yield good results within the definite limits already known.

Before penicillin in commercial form was released for civilian use, a number of such groups supplied themselves with working materials very effective for routine cases. Work upon this line was not approved by the official coördinator. Refusal to assist the hospital and the civilian in this way was widely condemned as an arbitrary abuse of power, hence was deliberately defied by a number of competent workers who believed that such service could have been rendered without delaying the actual development of

industrial production. With the release of industrially produced penicillin for civilian use, these temporary units will probably disappear.

In another proposal several layers of sterilized gauze were placed in a petri dish, flooded with culture solution, inoculated with *P. notatum*. The hospital group described the procedure and put forward the definite claim that after several days of incubation the whole mat removed from the dish, fastened over the injured area, and kept damp with the culture solution was effective. Although denounced by an official group as dangerous, the grade of workers responsible for this and some other proposals was fully equal to any in the penicillin field. Unfortunately, some popular publications assured people that making penicillin is easy, and requires neither special apparatus nor definite knowledge. Warnings of danger in such cases were amply justified.

Obviously there are two general uses for penicillin: (1) that of external application and (2) introduction into the blood stream. The highly purified industrial product if abundantly available may be used for both purposes but the field of external application offers a series of opportunities and problems not yet adequately covered by public information.

The part played by individuals and corporations in America in the development of penicillin has been deliberately omitted. Where so many individuals and financial interests have been involved and exchange of experimental results has been widely practiced under the pressure of a war program, an attempt to disentangle the contribution of the individual or the corporation toward the results obtained would be futile. It is equally doubtful to a discriminating spectator who has watched the development of practices and listened to the claims, whether any of the "patents applied for" honestly represent valid claims to originality.

Under the pressure of war needs the Office of Scientific Work and Development (OSRD) at Washington brought together those interested in manufacturing penicillin shortly after Florey and Heatley came to America in 1941. Contracts were prepared and signed with all prospective producers of penicillin. Each agreed to the pooling of the results of research and experiment. Coordinators representing the Government visited each laboratory

from time to time and conferences were held for the purpose of hastening production in every possible way. During this developmental period all penicillin produced was declared the property of the United States, to be paid for at specified prices per 100,000 Oxford units. Assignment of penicillin to particular groups covered chemical research, and special study of penicillin for specified groups of diseases. Penicillin was only released for individual civilian cases on appeal to the designated coördinator. The groups concerned in studying the chemistry of penciillin have been kept under contract of secrecy. Within the group, memoranda are marked "restricted." Even the culture media in use are on the list of restricted items. Recovery of penicillin is included in these restrictions. This paper sought to present the mycologist's story of penicillin discovery and development. The chemistry and industrial practices will be left to others.

During the three developmental years twenty-one producing units were approved by the authorities while many applications were not accepted. Something over $20,000,000 were reported to be invested in these industrial plants whose yield today is totalled in hundred billions of Oxford units per month. Most of the workers now concerned in penicillin production were entirely unacquainted with the kind of mold problems now before them, at the start. On the whole this is a remarkable accomplishment when we remember that the most careful surveys during the previous years had emphasized the small yield and instability of penicillin as rendering very doubtful its profitable production by any industrial process.

With penicillin now available through a thousand or more hospitals, it is in reach of emergency cases over the larger part of the United States. Its usefulness has been proved for whole series of diseases. Its limitations are being so carefully defined that its futile use where its failure becomes a calamity will be eliminated.

The successes of the penicillin program have stimulated research in the whole antibacterial field in the hope that supplementary antibiotic substances may be found to reach other needs equally urgent.

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NEW YORK

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Note: The literature of penicillin has been covered very fully in the publications of Merck, Squibb, and Winthrop, and in long series of research papers in the Journals of the last three years. No attempt at detailed citation is made here.

THE CUP FUNGUS, CIBORIA CARUNCULOIDES, PATHOGENIC ON MULBERRY FRUITS

H. H. WHETZEL[1] AND FREDERICK A. WOLF

(WITH 4 FIGURES)

INTRODUCTION

A cup fungus, first adequately described as *Sclerotinia carunculoides* (Siegler and Jenkins, 1922, 1923), parasitizes the fruits of white mulberry, *Morus alba* L., throughout the southeastern United States. As is well known, Prof. Whetzel was long a devoted student of disc fungi, especially those that are pathogenic to fruits. Such fungi, according to him, comprise the family Sclerotiniaceae. Furthermore in his unpublished notes the pathogen on mulberry, herein given consideration, is assigned to the genus *Ciboria,* which includes some of the most widely known members of this family.

The junior author's first contact with this organism on mulberry came in 1919 when he encountered it in the Sand Hills region of North Carolina. He next collected it in 1943 from trees grown for shade near Duke University. Here it has recurred abundantly during each of the three consecutive seasons covered by the present studies. This fungus is of such a nature that it undoubtedly will reappear year after year to attack the fruits of these trees.

Because of Prof. Whetzel's wide experience with Sclerotiniaceae, plans were laid in 1943 for a further study of this mulberry fungus. It was contemplated that the results eventually would be published conjointly, but as a consequence of the death of Prof. Whetzel the present report has perforce been prepared by the junior author.

[1] Professor Whetzel succumbed Nov. 30, 1944. His notes dealing with the fungus herein given consideration were made available through the courtesy of Dr. H. M. Fitzpatrick. I am indebted to Dr. Fitzpatrick, in addition, for his suggestions and criticisms in connection with the preparation of this report.

Even though Prof. Whetzel's notes, microscopic preparations, and other pertinent materials have been made available, it is fully realized that the value of this report would have been greatly enhanced had Prof. Whetzel lived to participate actively in its preparation.

HISTORICAL

The first account of this fungus is a statement by an anonymous author which is recorded in The Experiment Station Record (1903). This statement is merely a brief abstract of a paper read by W. A. Orton at a meeting of the American Association for the Advancement of Science. This anonymous abstract states: "W. A. Orton, in a paper read before Section G, described a disease of mulberry fruits which is reported from Georgia, Alabama, and Mississippi. Often as much as 50 per cent of the fruit is affected. The symptoms are peculiarly enlarged portions of the aggregate fruits. The disease is of fungus origin and the point of attack seems to be the seeds which are greatly enlarged. The fungus which is closely allied to *Gloeosporium* was described as a new genus, *Spermatomyces*, the species name *Mori* being given to it."

Apparently this paper by Orton was never published and the fate of the manuscript remains unknown. Curiously no further mention of the disease was made until 1920 when Taubenhaus (1921) published a semi-popular account in which he applied the appropriate descriptive name "popcorn disease." This account contains a brief description of the origin and development of the "sclerotium" and the extrusion of "colorless, roundish spores" in a "stout, gelatinous, whitish grey thread from the tip of each infected drupelet." Later (1937) he employed the name "mulberry swells," a designation that plant pathologists do not seem to consider so satisfactory a name as "popcorn disease." To date the studies by Siegler and Jenkins (1922, 1923) constitute the only ones that have been made from which an understanding of the developmental morphology of this pathogen can be gleaned, and the present purpose is to supplement their findings.

MATERIALS AND METHODS

The mulberry trees used as a source of materials were so located as to be easily accessible to the laboratory; consequently abundant

material was available at all times, an eventuality that greatly facilitated these studies. Progressive developmental changes in the pathogen were therefore followed closely. Some of the material collected for examination was appropriately fixed and was later embedded in paraffin, sectioned, and stained. In most cases Haidenhain's iron alum haematoxylin proved to be the best stain. Erythrosin, when used either alone or as a counterstain, was useful in tinting the gelatinous coating of ascospores and hyphae. However, equally well defined envelopes on ascospores are demonstrable by use of lacto-phenol cotton-blue. As an adjunct in interpreting structural features of sclerotia use was made of free-hand sections.

Pure cultures were prepared by isolation from sclerotia, apothecial tissue, and ascospores. Colonies of the pathogen were produced when tissues from sclerotia, that had been surface disinfected, were planted on agar plates. If apothecia are maintained in a humidor in such a way as to provide a high relative humidity, the ascospores are expelled in a "cloud" when the cover is removed. Accordingly advantage was taken of the liberation of ascospores *en masse* to entrap them on the surface of inverted poured agar plates and thus make isolations.

APPEARANCE OF THE DISEASE

Ciboria carunculoides, in common with many other members of the genus *Ciboria,* is a gynicolous species. In some instances only a few of the individual drupelets composing the syncarp may be involved and in others essentially all drupelets are stromatized. The common name, "popcorn disease," is unusually appropriate for this sclerotial disease for the reason that each mature stroma (sclerotium) bears a striking resemblance in size and shape to a grain of popcorn (FIG. 1).

Infection occurs at a time when mulberries come into flower. This conclusion was reached as the result of direct microscopic examination by Siegler and Jenkins (1923), and is supported indirectly by the fact that in nature the apothecia reach maturity and ascospores are forcibly discharged at this time. The disease is not evident, however, until three or four weeks after anthesis. It may be noted then, that some of the drupelets are larger than

FIG. 1. Popcorn disease of mulberry.

the remainder, and that sticky, grayish, columnar extrusions are prominently present at the tips of these affected drupelets (FIG. 3). Soon thereafter the sepals of healthy fruits are becoming fleshy whereas diseased ones remain firm and corneous. These differences become more marked as the time approaches for normal fruits to ripen. Mature stromatized drupelets are always considerably larger than healthy ones and are always grayish; *i.e.*, they never assume the color of mature normal drupelets.

The forcible expulsion of ascospores and their dissemination by convection currents is an efficient means of scattering the inoculum (ascospores), as shown by the distribution of diseased fruits throughout the trees. During each of the three seasons covered by these studies all the mulberries borne on the row of trees under observation were severely attacked. These trees are approximately 30 feet tall. Fruits borne on the lowermost branches were neither more nor less abundantly parasitized than those borne on the topmost branches. The abscission of diseased fruits occurs in late June and early July; *i.e.*, at the same time that the healthy ripe fruits are being shed from disease-free trees growing a few blocks away.

MORPHOLOGY AND DEVELOPMENTAL HISTORY OF THE PATHOGEN

Ciboria carunculoides, in common with all other members of the genus, lacks a conidial stage. Its developmental cycle consists of two phases or stages, a sclerotial and an apothecial. Its sclerotial stage functions for hibernation and for the initiation and nurturing of developing apothecia. The apothecial stage (FIG. 2) functions for reproduction and dissemination. The former stage is initiated in the vicinity of Durham, N. C., from ascospores that are discharged during late March and early April. The latter is initiated about a month later. Each stage requires for its complete development a period of approximately eleven months duration.

Sclerotial stage: The sclerotia are stromatic structures that are constituted both of fungus and of suscept tissues. These stromata have their beginning at the time of flowering. From sections of flowers it may be noted, as was done by Siegler and Jenkins (1923), that hyphae arising from ascospores ramify throughout

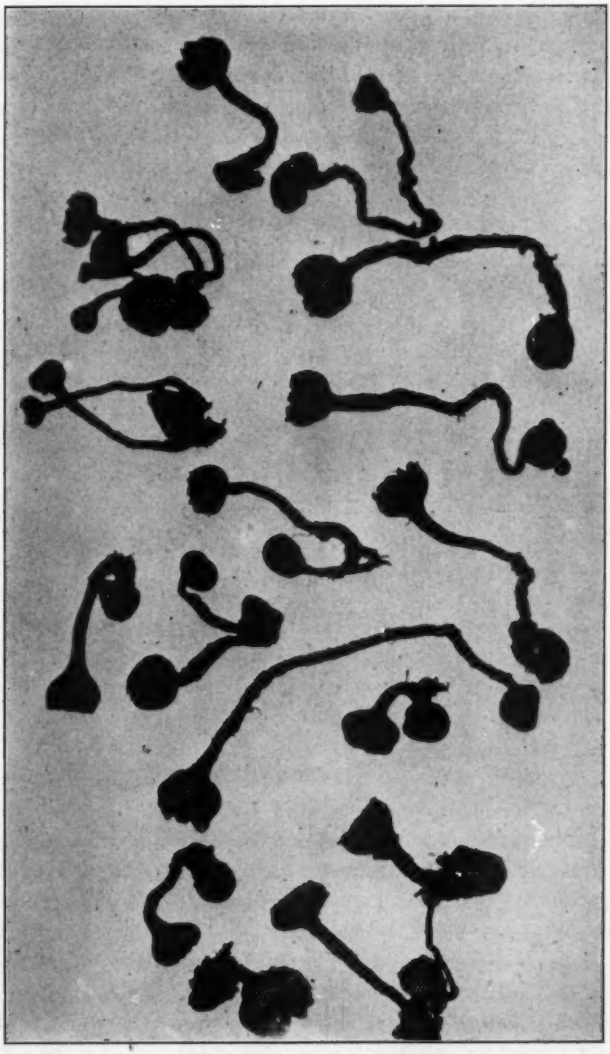

FIG. 2. *Ciboria carunculoides.*

the tissues of the stigma and style (FIG. 4 A). It is not until three or four weeks later, however, that morphologic symptoms are apparent. Then each infected drupelet is larger than normal, is urceolate in shape, grayish white, and of firm texture. As seen under the microscope, the exterior of each stroma is found to consist of the outer tissues of the sepals (FIG. 3). Immediately beneath is an hymenial layer that practically envelops the entire

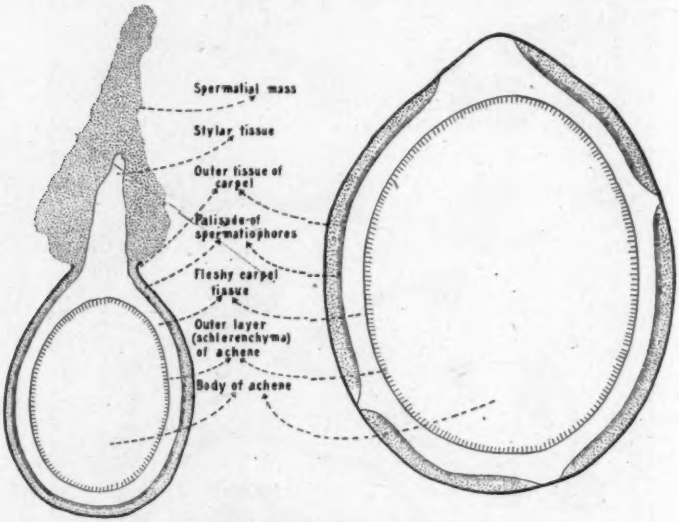

FIG. 3. Popcorn disease of mulberry.

stroma. This layer (FIGS. 3 and 4 C) is constituted of a palisade of spermatiophores and interspersed archicarps. Globular spermatia are budded *seriatim* from the apices of the spermatiophores, and are extruded in a gelatinous matrix as a collar around the remnants of the style and stigma. They are produced in such profusion as to form columns one to two millimeters in height (FIG. 3). The mycelium appears to course at will, both intracellularly and intercellularly, throughout the style and stigma tissues (FIG. 4 A), and is for the most part densely compacted and intercellular within the basal portions of the carpels (FIG. 4 B).

The layers of sclerenchyma that constitute the outer portion of the achene are quite free from invasion (FIGS. 3 and 4 D), and

consequently remain quite unchanged even in mature sclerotia. The presence of sclerenchyma makes difficult the preparation of satisfactory paraffin sections. All tissues on the interior of the achene are densely and compactly occupied by intracellular fungus tissue (FIG. 4 E). Here each hyphal element is invested with a gelatinous membrane.

Intercellular hyphae sparsely occupy the bracts that subtend each drupelet. These bracts seem never to be destroyed and they are never incorporated into the sclerotia. Early in June the grayish sclerotia will have become ellipsoidal and 3 to 5 millimeters in longest diameter (FIG. 3). They may then shatter singly or the entire syncarp *in toto* may fall away. The fallen sclerotia soon become black, are of a corneous consistency, and remain unchanged in size during dormancy. Such sclerotia as are not destroyed by biological or other agencies during the fall and winter produce apothecia in late March and April of the following year.

As a first step leading to the breaking of dormancy the sclerotia swell and may become several times their size while dormant. Soon thereafter one or two stipitate apothecia arise from each swollen sclerotium (FIG. 2). Efforts to break dormancy of stored sclerotia prematurely, by modification of temperature and moisture conditions, have been unsuccessful.

Apothecial stage: As previously stated, apothecial development is initiated about a month after the young fruits have been inoculated. In all likelihood the developmental pattern in essential features is like that described by Drayton (1934) for *Sclerotinia Gladioli* (Massey) Drayton. From the spermatiophores arranged in palisade fashion, spermatia are acrogenously abstricted throughout a period of approximately three weeks duration. Slender deeply-staining hyphae, that are indicated to be tips of archicarps (trichogynes), are interspersed among the spermatiophores, and they extend well above them. Spermatia have been found attached to these trichogyne-like structures, but it has not been possible to determine whether fusion of spermatium with trichogyne actually occurs. It seems very probable, in the light of knowledge of other disc fungi, that they do and that fertilization follows. During the entire period thereafter until the following spring, little further change occurs. Then follows within a brief period the swelling

of sclerotia, protrusion of stipes, and expansion of the discs. Mature cupulate discs are 4–12 mm. in diameter and brown in color. The cylindrical stipes are straight or flexuous, attenuated downward, 15–42 mm. long, and brown. The cylindrical asci measure 104–123 × 6.4–8 μ. The ascospores are reniform, each with a peculiar structure, the caruncle, on the concave side, and they measure 6.4–9.6 × 2.4–4 μ. The filiform paraphyses are usually branched and septate (FIG. 4 G).

The genus *Ciboria,* as delimited by Whetzel in unpublished notes, has the following characteristics: The stromata (sclerotia) are black or brown, andricolous or gynicolous, and mummioid. The spermatiophores form a mantle around the developing sclerotium, and the spermatia are globose or ovate, hyaline or faintly brownish in mass. A conidial stage is wanting. The apothecia are cupulate to shallow saucer-shaped, becoming flat expanded or even reflexed convex, and are some shade of brown, especially vinaceous brown. They are small to medium in size. The ascospores are ellipsoid, inequilateral, 1-celled, hyaline, smooth or adorned with stipples or depressions.

The type species is *Ciboria Caucus* (Reb.) Fuckel, Symb. Myc. 311, 1869.

Manifestly the mulberry pathogen should be referred to *Ciboria* consequently an emended brief description of it, together with essential notes on exsiccati, follows:

Ciboria carunculoides (Siegler and Jenkins) Whetzel, comb. nov.

Stroma (sclerotium) consisting of closely compacted hyphae with thick gelatinous walls together with remnants of the fleshy sepals and of the ovarian tissues; urceolate, when young, enclosed by a whitish outer membrane of the calyx, 3–5 mm. diam.; globose to subspherical and black when mature, 7–10 mm. diam. Rind constituted of several layers of dark brown cells. Medulla of densely interwoven hyphae, both inter- and intracellular.

Spermatia hyaline, ovate, 2–4 × 2–2.3 μ, av. 2.8 × 2.5 μ (Siegler and Jenkins), 3.6 × 2.4 μ (Whetzel), produced successively from the tips of slender, obclavate spermatiophores which form a continuous hymenium over the surface of the developing sclerotium. Spermatia exude in waxy threads or masses through the tip of the membrane that encloses the developing sclerotium.

Apothecia one to several from a sclerotium; disc cupulate to subcupulate, 4–12 mm. diam.; snuff brown within, Prout's brown without. Stipe cylindrical flexuous, smooth with tufts of anchoring rhizoids, attenuated downwards, 15–42 mm. in length by 1.5 mm. in diam., Prout's brown. Asci cylindrical to cylindro-clavate,

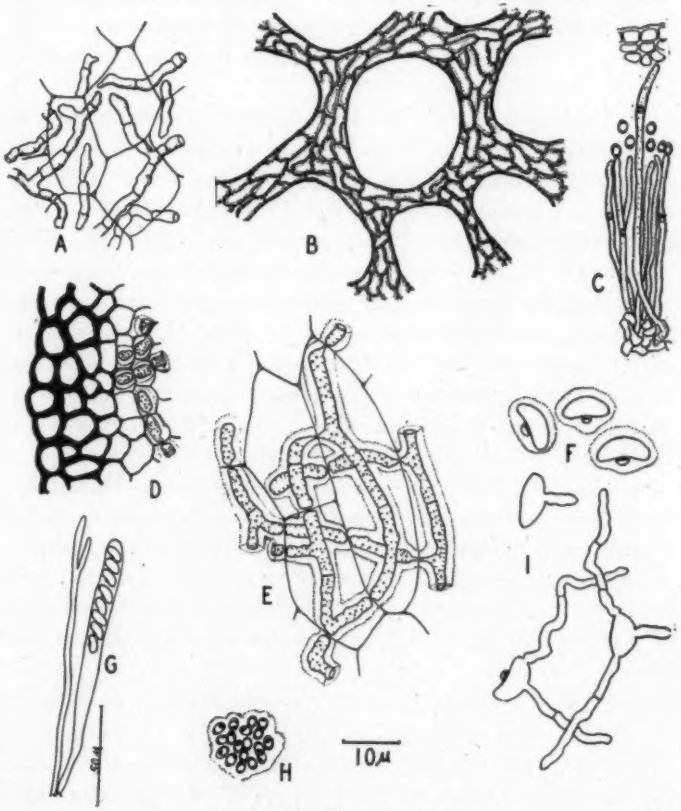

FIG. 4. *Ciboria carunculoides.*

104–123 × 6.4–8 μ, av. 117 × 7 μ, 8-spored. Ascospores uniseriate, reniform, hyaline, 6.4–9.6 × 2.4–4 μ, av. 7.6 × 3.1 μ, with two bodies constituting the caruncle on the concave surface; *i.e.*, the one more or less rhombic as seen from above, 2 × 4 μ, and the other adjoining it, more or less hemispherical, 3 μ in longest diam. Paraphyses filiform to cylindro-clavate, simple or branched, usually septate, 94–128 × 1.8–2 μ.

On fruits of cultivated *Morus alba* L.

Distribution: [1] Alabama, Arkansas (Dunegan and Allen, 1939), Georgia, Florida, Louisiana, Mississippi, North Carolina and South Carolina (Jenkins and Siegler, 1938), and Texas.

Type specimen: Collected by E. A. Siegler, at Scranton, S. C., April 4, 1922; deposited in the Mycological Collections, U. S. Dept. Agr. Duplicate (apothecia), Cornell Univ. Plant Path. Dept. (C. U. P. P.) No. *11810.*

Icones: Siegler and Jenkins, Jour. Agr. Res. **23**: text-fig. 1, and Pls. 1 and 2, 1923.

Other specimens examined: C. U. P. P. No. *33598* (apothecia and sclerotia), apothecia developed in Washington, D. C., by Jenkins, from sclerotia collected in type locality, March 21, 1923; No. *34072* (apothecia). Apothecia developed in Washington, D. C., by E. A. Siegler, from sclerotia collected at Scranton, S. C., March and April 1924 (Myc. Coll. U. S. D. A. No. *68334*); No. *33599* (young sclerotia), Meridian, Miss., June 6, 1927, collected by L. D. Walker; No. *33600* (apothecia), Clemson College, S. C., June 1928, collected by L. M. Fenner; No. *33939* (young sclerotia), Durham, N. C., May 1944, collected by F. A. Wolf.

According to Dr. Anna E. Jenkins the Mycological Collections, U. S. Dept. Agr. contains a specimen on which Orton based his original study labelled "*Spermatomyces Mori* Orton, on *Morus,* Sylvester, Ga., Apr. 29, 1903, Orton," and another from the Herbarium of C. L. Shear labelled "*Sclerotinia carunculoides,* on fruit of mulberry, *Morus,* Delchamps, Ala., coll. L. J. Delchamps, May 1902."

Abundant specimens of sclerotia and apothecia from Durham, N. C., have been deposited in the Farlow Herbarium, Harvard University, in the Herbarium of the New York Botanical Garden and in the Herbarium of the Department of Plant Pathology, Cornell University.

Cultures: Potato dextrose agar, agar enriched by inclusion of green mulberry fruits, and sterilized mulberry fruits alone have

[1] Records of collections are contained in Plant Disease Reporter **8**: 129, 1924; **10**: 12, 1926; **15**: 68, 101, 1931; **19**: 61, 1935; and in Suppl. **20**: 117, 1922; **28**: 375, 1923; **47**: 281, 1926; **52**: 93, 1927; **81**: 88, 1931; **96**: 173, 1936; **119**: 182, 1940; **128**: 311, 1940; **131**: 48, 1941.

been employed as media. Tissue plantings from sclerotia have been made at various times throughout the year. No particular difficulties attend isolation except that the organism does not grow rapidly on any of these media. The colonies are white and floccose but lack distinctive features except that sclerotial aggregates may form after approximately a month. Such sclerotia have not been induced to form apothecia however.

GENERAL CONSIDERATIONS

There are a number of points regarding *Ciboria carunculoides* that seem worthy of special attention. Not the least among these is the gelatinous envelope that so prominently invests hyphal elements within sclerotia and ascospores. The senior author has noted that ascospores of most (presumably all) species of Sclerotinaceae possess a gelatinous covering which is lost if specimens are dried or are preserved in fluids. Seemingly no one has directed attention to such envelopes nor have they been illustrated and described in any accounts that have come to the notice of the junior author. Interestingly the ascospores of *Monilinia fructicola* (Wint.) Honey (*Sclerotinia fructicola* (Wint.) Rehm), occurring on plums, are found to have very prominent envelopes. This gelatinous membrane, in the case of *C. carunculoides,* is strikingly apparent while the ascospores are still within the asci and even after they have been freshly liberated. It is not present in preserved specimens nor can the caruncles be found after preservation. The fact that both structures disappear during preservation may be interpreted to indicate that they are of the same nature and that the caruncles may function in gland-like fashion to generate the envelope.

Undoubtedly the gelatinous covering of ascospores serves this fungus in two ways that are essential for its existence: (a) it causes the ascospores to adhere to the suscept and (b) it both retains and supplies moisture during germination. Without this device infections might be limited to periods of high relative humidity, whereas with an ever-available supply of moisture, germination of ascospores and initiation of infection need not be inhibited even during weather when low relative humidities prevail. The increase in size of sclerotia, which occurs with breaking of dor-

mancy shortly before the expansion of apothecia, is not a result of growth but of swelling of fungal cells from absorption of moisture by the gelatinous coating. The sclerotia thereby become water storage reservoirs. Their water supply becomes useful for conversion of food reserves and for maintenance of turgor during the critical period of ascospore expulsion. In the light of these interpretations, therefore, gelatinous membranes constitute an adaptive device of great ecologic significance for *C. carunculoides*.

Attention may well be directed to pecularities in the range of this fungus. It is apparently restricted to the southern parts of the United States (Jenkins and Siegler, 1938) although its host, *Morus alba*, indigenous to China and Formosa, has been widely planted throughout the eastern United States from Canada to the Gulf of Mexico. Why has *C. carunculoides* not appeared farther northward? Its restricted range becomes the more puzzling since most of the known species of *Ciboria* have been collected only in the colder regions of the North Temperate Zone. If *C. carunculoides* is indigenous to the southern United States, how could so striking a fungus have been overlooked by such mycologists as Earle, Atkinson, Carver, and Tracy, who collected so assiduously during the latter part of the 19th century in the region in which this organism is known to occur? It seems a reasonable presumption that this fungus must have been introduced quite recently from China.

Evidence that it is native to China and was introduced into the United States is, however, not convincing. As bearing on introduction from the Orient, consideration should be given to the possible identity of *C. carunculoides* and *C. Shiraiana*, the latter having been described as *Sclerotinia Shiraiana* by Hennings (1900) from specimens on mulberry fruit sent from Japan by Shirai. It can scarcely be questioned, from the records by Teng (1934, 1939) and by Tai (1937) in China, and by Sawada (1937) in Formosa, that *C. Shiraiana* occurs in Asia. Incidentally Sawada records the occurrence of *C. Shiraiana* both on *Morus alba* and *M. acidosa* Gr. It should be remembered, however, that collectors throughout Asia have failed to recognize *C. carunculoides* among their specimens. Siegler and Jenkins (1923), after examining specimens sent to them by Shirai, were convinced that

C. Shiraiana and *C. carunculoides* are specifically distinct. They pointed out that the drupelets are collectively merged into one stroma that simulates a mumified fruit in the former species, while in the latter each drupelet is separately stromatized. Moreover the ascospores of the former are ovoid to ellipsoid and not reniform as in the latter. According to Teng (1939, p. 171) the asci of *C. Shiraiana* are 140–170 × 8–11 μ and the ascospores are 11–15 × 4.5–6 μ. These measurements are in excess of those given in the original description by Hennings. Moreover the measurements both of Hennings and of Teng show that the asci and ascospores of *C. Shiraiana* are larger than those of *C. carunculoides*. On the other hand the external characteristics of apothecia of the two are strikingly alike. Nevertheless there can be little doubt that the two species are distinct. Judging from Prof. Whetzel's notes, he was of this opinion and was convinced that the Asiatic mulberry fungus belongs to *Ciboria* and should become **Ciboria Shiraina** (Hennings) Whetzel, comb. nov.

SUMMARY

A developmental study has been made of a disc fungus that attacks the fruits of *Morus alba*. From the results obtained, the organism is transferred from *Sclerotinia* to *Ciboria*, and is assigned the binomial *Ciboria carunculoides* (Siegler and Jenkins) Whetzel.

This fungus possesses a sclerotial phase and an apothecial phase, but lacks conidia. Its ascospores, which are forcibly expelled, lodge on the stigmas and constitute the inoculum for initiation of infection at the time of flowering. As a result each drupelet of the aggregate fruit may become transformed into a separate sclerotium.

Sclerotia are composed both of fungus and of suscept tissues and have somewhat the appearance of grains of popcorn.

Apothecia for the succeeding year are initiated in the spring about a month after discharge of ascospores. They originate from elements of a mantle that occurs immediately beneath the outer tissues normally destined to become the fleshy portion of the mulberry fruits. This mantle completely invests the young sclerotium and consists of spermatiophores with interspersed archicarps.

The spermatia are produced in such abundance as to be extruded in a column at the tip of each sclerotium.

Sclerotia fall to the ground during mid-summer, become black, and remain dormant until the following spring. Then each bears one or two apothecia. The breaking of dormancy is first indicated by increase in size of sclerotia. Increase in size is accounted for by the presence of a gelatinous covering on the sclerotial hyphae. This gelatinous envelope absorbs water, causing the sclerotia to swell, and also functions to maintain turgor during expulsion of ascospores.

Ascospores possess thick gelatinous envelopes which cause them to adhere and provides moisture for germination.

An Asiatic fungus, *Sclerotinia Shiraiana*, parasitic on mulberry appears to be distinct from *C. carunculoides* but properly belongs in the same genus. It is herein transferred to *Ciboria Shiraiana* (Henn.) Whetzel.

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EXPLANATION OF FIGURES

FIG. 1. Stromatized fruits of mulberry, the sclerotial phase of *Ciboria carunculoides*. Each drupelet may become a sclerotium.

FIG. 2. Apothecial phase of *C. carunculoides*, arising from sclerotia.

FIG. 3. Diagrammatic representations to the same scale of stromata and their components in section. At the left a young sclerotium when first the fruits are observed to be diseased; at the right a sclerotium at the stage when sclerotia are shattering and falling to the ground.

FIG. 4. Detailed sketches of parts shown in figure 3. All figures except G are drawn to the same scale (near H): A, The mycelium courses through the thin-walled tissues of style and stigma; B, Within the fleshy tissue of the carpel the mycelium is mainly intercellular; C, Palisade of spermatiophores beneath the outer carpel tissues. An occasional deeply-staining hypha, presumably the upper portion of an archicarp, extends beyond the spermatiophores; D, The sclerenchyma tissue at the exterior of the achene is quite free from invasion. The cells beneath are occupied by hyphae with gelatinous sheaths; E, Tissue from the central portion of the achene occupied by sheathed hyphae; F, Freshly discharged ascospores of *C. carunculoides;* G, Ascus and branched paraphysis; H, Mass of spermatia embedded in a mucoid matrix, from the column extruded around the style and stigma; I, Germinating ascospores.

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A LEAF SPOT OF TALL FESCUE CAUSED BY A NEW SPECIES OF CERCOSPORA [1]

JOHN R. HARDISON [2]

(WITH 1 FIGURE)

During an investigation of grass diseases in Kentucky in 1942–44 a leaf spot was collected on *Festuca elatior* var. *arundinacea* (Schreb.) Wimm. caused by a fungus tentatively identified as *Cercospora* sp.[3] Specimens were sent to Dr. Charles Chupp, Cornell University, who replied that the fungus was distinct from any of the species of *Cercospora* described on the Graminaceae, especially by its distinctly acicular and long conidia and also by its almost straight conidiophores. Dr. Chupp very kindly suggested the name *Cercospora Festucae* Hardison and sent detailed morphological notes which largely represent the description below.

Cercospora Festucae Hardison, sp. nov.

Maculis ovatis vel elongatis, 0.5–4 mm. longis, centro cinereis, margine purpurea, absque stromatibus vel cellis paucis brunneis; fasciculis cum 2–8 conidiophoris divaricatis; conidiophoris prope bases pallidis vel mediocriter olivaceo-brunneis, pallidioribus et aliquando angustus ad apicibus, parce septatis, rarus geniculatis, prope rectis, non-ramosis, apicibus rotundatus vel subtruncatis, 3.5–5 × 50–800 μ; conidiis hyalinis, acicularibus, curvatis vel undulatis, indistincte pluriseptatis, infra truncatis, supra acutis, 2–4 × 40–300 μ.

In foliis vivis *Festucae elatiorae* var. *arundinaceae*. Specimen typicum legit J. R. Hardison in foliis *Festucae* prope urbem Lexington, Kentucky, July, 1944.

[1] Coöperative investigations between the Division of Forage Corps and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Published with the approval of the Director of the Oregon Experiment Station as Tech. Paper No. 455. Contribution of the Department of Botany.

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[3] Hardison, John R. Observations on Grass Diseases in Kentucky, Sept. 1942–44, and a Preliminary Check List. Plant Dis. Reptr. **29** (3): 76–85. 1945.

Leaf spots oval to elongate, 0.5–4 mm. in length, gray center, purplish border; stromata none or a few brown cells; fascicles 2–8 divergent stalks; conidiophores near base pale to medium olivaceous brown, paler and sometimes more narrow toward the tip, sparingly septate, rarely geniculate, almost straight, not branched, rounded to subtruncate tip, 3.5–5 × 50–800 μ; conidia hyaline, acicular, curved or undulate, indistinctly multiseptate, base truncate, tip acute, 2–4 × 40–300 μ.

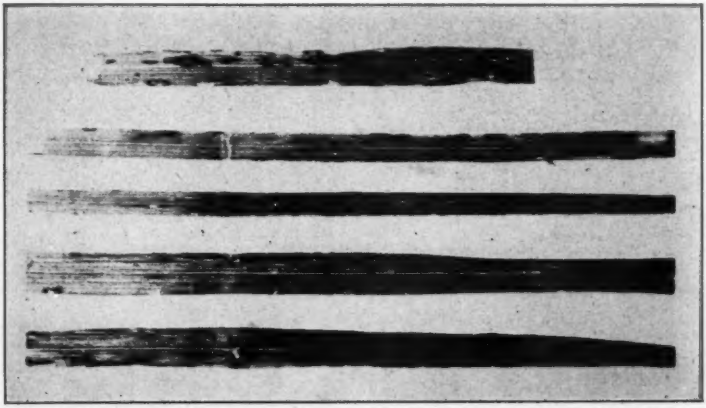

FIG. 1. Habit of *Cercospora Festucae* on leaves of *Festuca elatior* var. *arundinacea*.

In living leaves of *Festuca elatior* var. *arundinacea*. Type specimen collected in the vicinity of Lexington, Kentucky, July 1944, John R. Hardison.

Type material has been deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland (*B.P.I. 71419*) and in the herbarium of Oregon State College (*O.S.C. 15,094*).

The occurrence of a *Cercospora* leaf spot on tall fescue, *F. elatior* var. *arundinacea*, is of considerable interest since very few diseases have been observed on this grass. The effects of the disease have been mild thus far. Typical symptoms are shown in figure 1. It will be interesting to see if it increases in severity or in occurrence.

A considerable number of plants of common meadow fescue, *Festuca elatior* L., were growing nearby, and none of these were observed to be infected. However, several plants intermediate in type between *F. elatior* and *F. elatior* var. *arundinacea* were infected, and possibly *F. elatior* will be found to be susceptible.

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A NEW SPECIES OF CEPHALOSPORIUM CAUSING PERSIMMON WILT

BOWEN S. CRANDALL[1]

(WITH 1 FIGURE)

In the studies of the wilt disease of persimmon (1) in the Southeastern States a fungus was isolated from the wood of wilting persimmons and found fruiting in abundance on wilt-killed trees. It is a species of *Cephalosporium* (FIG. 1).

A search of the literature disclosed no member of the genus *Cephalosporium* resembling the persimmon wilt fungus. The literature on diseases of persimmon and ebony contains no reference to a disease caused by a *Cephalosporium*. In the course of the investigation of this disease no perfect stage has been found, nor has it been possible to produce one by intercrossing all available isolates collected during the scouting activities from all parts of the known range of this fungus. Little variation has thus far been observed between any of the isolates, regardless of their origin. For this reason, in the description that follows, all measurements are based on the isolates of the fungus from the point of original discovery in Tennessee. The specimen and culture, No. D-27, on which the description is based, have been designated as the type and deposited in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture. The fungus causing the wilt disease is here described as a new species. Description of the hyphae is based on the growth of the fungus on 2 per cent malt agar.

Cephalosporium Diospyri sp. nov.[2]

Mycelium in culture at first appressed and watery, later faintly pinkish white and fluffy; aerial mycelium often composed of many

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[2] The Latin description was prepared by Edith K. Cash, Associate My-

parallel adherent hyphae that are hyaline, vesiculose, sparsely branched, 3.8 μ to 7.6 μ in diameter with indistinct septations; conidiophores hyaline, simple, non-septate, 1.5 μ to 3.5 μ wide at the base, gradually tapering to the tip, 10 μ to 60 μ long, mostly arising at right angles from the hyphae; conidia hyaline when viewed singly but orange-pink [3] in mass, continuous, ovate or ellipsoid to cylindric, the ovate predominating in young cultures and in nature, 2.7–11.7 $\mu \times$ 1.8–5.4 μ, but 94 per cent 2.7–4.5 μ long, produced acrogenously and forming small globose heads averaging 10 μ in diameter, which break up readily in air or water.

Mycelium in culturis primo appressum aquosumque, deinde pallide roseolo-album et lanosum; mycelium aerium ex hyphis multis, adhaerentibus, hyalinis, vesiculosis, parce ramosis, 3.8–7.6 μ in diam., indistincte septatis saepe conpositum; conidiophora hyalina, simplicia, eseptata, e basi 1.5–3.5 μ lato apicem versus gradatim attenuata, 10–60 μ longa, plerumque ad angulos rectos ex hyphis oriunda; conidia singula hyalina, in massis aurantio-rosea, continua, ovata vel ellipsoideo-cylindrica, in culturis juvenilibus et in natura praecipue ovata, 2.7–11.7 μ longa, 1.8–5.4 μ lata, maxima ex parte (94 per centum) 2.7–4.5 μ longa, acrogena, in capitulis globosis circum 10 μ in diam. in aquo et aere mox dissolventibus aggregata.

Cephalosporium Diospyri differs from most other described members of this genus in its abundant production of orange-pink spores and in having a faint pink color in culture. Two species, *C. acremonium* Cda. and *C. carpogenum* Ruehle, are described as being faintly pink in culture. *C. acremonium* Cda., as described by Corda (**2**), was given such wide limits that it would include almost any *Cephalosporium*. Reddy and Holbert (**6**) summarized the later concepts of this species, which are in general agreement in giving to this species a spore size averaging 4.5 $\mu \times$ 1.3 μ. The isolates of *C. acremonium* Cda. with which they worked had a spore size of 3.6 $\mu \times$ 1–1.8 μ and an average size of 4.3 $\mu \times$ 1.3 μ. *C. carpogenum* Ruehle (**8**) is described as having spores 4–8.5 $\mu \times$ 1.4–2.8 μ. *C. Diospyri* may be differentiated from either *C. acremonium* or *C. carpogenum* by its more ovoid spores and by the larger size of the ellipsoid spores present.

cologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[3] Color according to Ridgway (**7**).

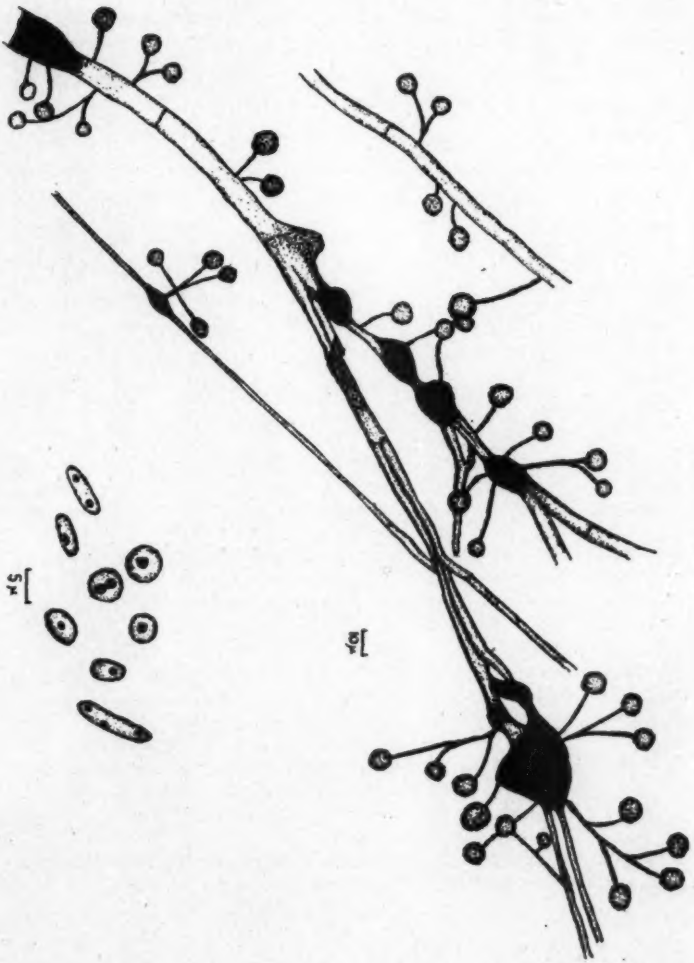

FIG. 1. A, Hyphae and conidiophores; B, Conidia of the persimmon wilt *Cephalosporium*.

The fungus has been found in nature on the American persimmon (*Diospyros virginiana* L.) on which it causes a serious wilt disease; on the Oriental persimmon (*D. Kaki* L. f.), which is nearly immune but is often killed when grafted on susceptible *D. virginiana* roots.

By inoculation it has been found that *D. ebenaster* Retz., a widely cultivated East Indian species of which the material came from Mexico, is highly susceptible; *D. texana* Scheele of Texas and Mexico is fairly susceptible; *D. lotus* L. used as grafting stock in the Orient is very slightly susceptible. Inoculations have failed on *D. Rosei* Standley of Mexico, *D. montana* Roxb. of India, and *D. discolor* Willd. from the Philippines.

To date the disease is known to occur in the United States in Texas (**5**), Tennessee, Mississippi, Alabama, Georgia, Florida, South Carolina, and North Carolina (**3, 4**).

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PRESERVATION OF MOLDS BY THE LYOPHIL PROCESS

KENNETH B. RAPER[1] AND DOROTHY F. ALEXANDER[2]

(WITH 3 FIGURES)

Subsequent to the establishment of the four Regional Research Laboratories by the Department of Agriculture, a large collection of industrially important microörganisms was assembled at the Northern Regional Research Laboratory as an integral part of the research program of the Fermentation Division. From the outset it was recognized that this collection could be of the greatest possible value only if variation in the organisms was kept at an absolute minimum. It was likewise recognized that the routine labor involved in propagating individual cultures should be reduced as much as possible in order that a large and varied collection could be maintained. The accomplishment of both objectives through preservation of cultures by some type of vacuum desiccation from the frozen state appeared as a real possibility. The experience of numerous bacteriologists (Shackell, **1909**; Hammer, **1911**; Rogers, **1914**; Swift, **1921**, **1937**; Brown, **1932**; Elser, Thomas, and Steffen, **1935**; and Flosdorf and Mudd, **1935**, **1938**) during the past quarter of a century left little doubt that this method could be successfully used for the preservation of bacterial cultures contained in the collection. Some published reports (Rogers, **1914**; Elser, Thomas and Steffen, **1935**) and other work then known to be in progress (Wickerham and Andreasen, **1942**) likewise indicated that the method could probably be successfully applied to the yeasts. Regarding the molds, however, there were few and fragmentary reports, and these were generally not too

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favorable. Bushnell (**1941**) reported unsuccessful attempts to preserve fungi (identity unstated) by freezing and by subsequent vacuum desiccation. Conant in 1941 (personal communication) reported rather indifferent success. Among the strains tested by Wickerham and Andreasen (**1942**) were 16 "Dermatophytes and other molds" of which 15 were viable at 12 months. Included in this number were 2 species of *Aspergillus*, 2 of *Penicillium*, various pathogenic species, and some miscellaneous forms.

Despite a dearth of positive evidence it was decided to investigate the possibility of preserving molds by this technique, and to process the entire mold collection if it could be demonstrated that the method was applicable to the fungi. Preparations of a limited number of selected cultures were made during the spring and early summer of 1941. In December of the same year, when the preparations had been stored for approximately 6 months, streak plates made from the dried cultures showed neither appreciable loss of viability nor apparent change in morphological characteristics. While these results were in no sense conclusive, they were at least indicative, and the contemplated program of mass freezing and drying was pushed through to completion as rapidly as possible. By July 1, 1942, the entire collection of 1850 molds, more than 900 yeasts, and approximately 400 bacterial cultures then contained in the Collection at the Laboratory had been processed. The present paper is concerned with results obtained with preservation of molds up to the present time and is presented in response to an increasing number of inquiries concerning the practicability and reliability of this method for the preservation of mold cultures. These inquiries have been particularly numerous regarding the preservation of penicillin-producing strains of *Penicillium notatum* and allied species. Special attention will therefore be given to these molds.

FIG. 1. Materials and preparation. A, plate culture of *Penicillium chrysogenum*, NRRL 1951.B25, from which spores (conidia) have been removed; B, platinum loop, 4 mm., used to collect spores; C, spore suspension in sterile beef serum; D, long, thin-necked pipette used for filling lyophil tubes; E, lyophil tubes labeled, containing approximately 0.05 cc. of spore suspension each, ready to be processed; F, completed preparations showing compact chalky pellets of dried, spore-laden serum.

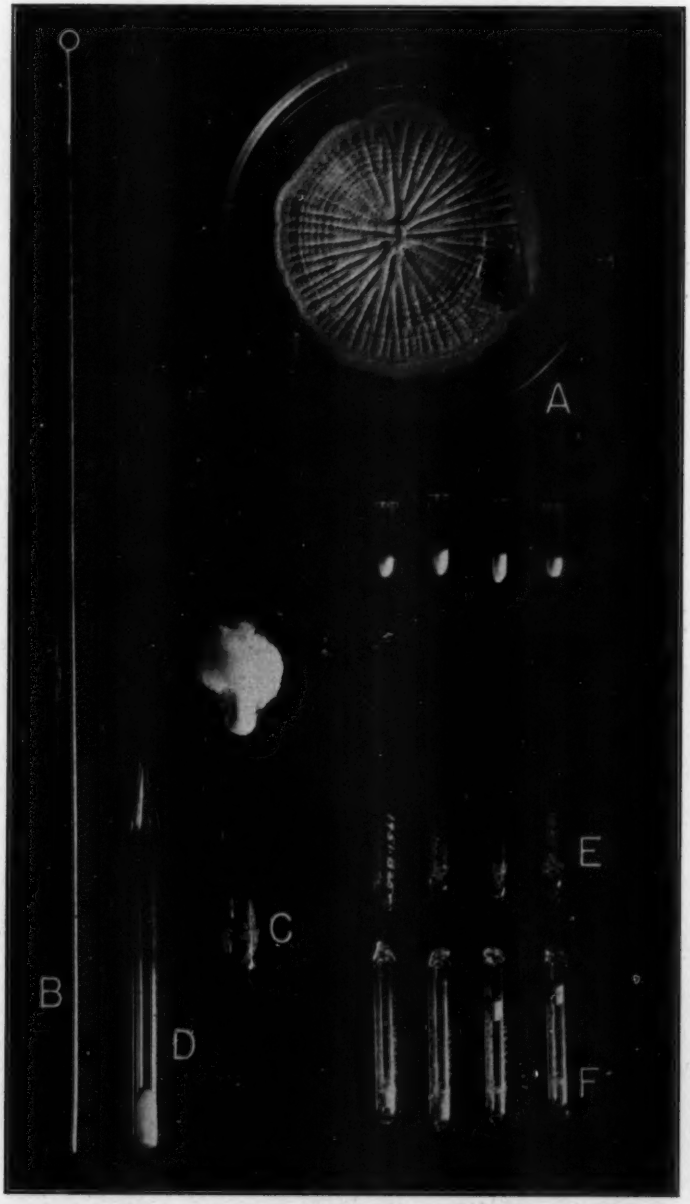

FIG. 1.

APPARATUS AND METHODS

The present report is based upon cultures that were processed on three pieces of lyophil apparatus. Cultures reported as having been maintained in lyophil form for 21 months or more were lyophilized according to the method used by Wickerham and Andreasen (1942) and upon an apparatus of the type described and illustrated by them. Those reported as having been kept in lyophil form for less than 21 months were processed on the apparatus shown in figure 2, which was designed by Dr. L. J. Wickerham and constructed in the mechanical shops of the Northern Regional Research Laboratory. Cultures preserved within the past year have been processed on the table model shown in figure 3. The methods employed were essentially the same for each type of apparatus and will be briefly described in connection with the operation of the portable unit shown in figure 2.

Materials used to prepare the cultures for drying include: (1) plugged and sterilized agglutination tubes; (2) micropipettes made from 10-mm. Pyrex glass tubing; (3) plugged and sterilized lyophil tubes made of 4-inch lengths of 6-mm. Pyrex glass tubing, sealed at one end and lightly fire-polished at the lip; and (4) glass writing ink [3] for marking the tubes.

Cultures were grown, in the usual manner, on Czapek's solution agar or malt extract agar in either test tubes or Petri dishes (FIG. 1 A) for from 1 week to 10 days, or until a sufficient quantity of conidia was produced. Approximately 0.25 cc. of sterile beef serum was placed in an agglutination tube, and spores or conidia were added from the culture to be lyophilized until the resulting suspension was comparatively dense (FIG. 1 C). By means of a micropipette (FIG. 1 D), approximately 0.05 cc. of the suspension was then dispensed into each of four previously labeled, sterile lyophil tubes. The cotton plugs were replaced, and the excess cotton was burned off. The remaining portion of the plug was then pushed down into the tube to a depth of about one-half inch as a precaution against possible contamination during processing, and to prevent the cotton from being drawn up into the apparatus during evacuation (FIG. 1 E).

[3] We have employed successfully a product marketed by the Clay-Adams Co. of New York under the trade name "Gold Seal" Laboratory ink.

The lyophil tubes containing the spore suspension were then attached to the manifold by inserting them in the rubber sleeves shown in figures 2 and 3. The whole manifold, the height of which can be adjusted by a screw-lifting device, was lowered, and the ends of the tubes containing the spore suspension were submerged in a bath of dry ice and methyl cellosolve at a temperature of about — 40° to — 50° C. The small amount of material in the tubes was completely frozen within a few seconds, and evacuation by means of a vacuum pump [4] was begun as soon as this was accomplished. The tubes were then raised above the surface of the bath to a position where the temperature at the level of the frozen suspension was approximately — 10° C.; they were held at this temperature by adjusting the height of the manifold during the drying process.

In the portable apparatus (FIG. 2), as in the smaller apparatus developed by Wickerham and Andreasen (**1942**), water vapor removed from the frozen preparations is taken up in a column of anhydrous calcium sulfate (Drierite [5]). This column, which is placed between the manifold and the vacuum pump, is provided with two glass stopcocks, one at the lower end, leading to the pump, and another in the rubber hose, leading to each manifold. The column can thus be closed off and the desiccant protected when the apparatus is not in operation. At the end opposite that of attachment to the drying column, each manifold is connected to a single, Bruner-type vacuum gauge by means of a two-way glass stopcock. The two manifolds can be operated simultaneously, or either manifold can be operated alone. Employing both manifolds, a total of 60 preparations can be desiccated in one operation. When the apparatus is fully loaded, desiccation usually requires 1½ to 2 hours.

At the outset, the frozen suspension normally appeared glassy, but, as drying progressed, the mass became chalky in appearance and assumed the shape of a compact, cylindrical pellet. When the pellets appeared completely dry, the tubes were raised to room

[4] Cenco Hi-Vac and Welch Duo Seal pumps (FIGS. 2, 3) have been used successfully.

[5] Trade name of a commercial product marketed by the W. A. Hammond Company, Yellow Springs, Ohio.

temperature, the bath was covered, and drying was continued for half an hour to insure thorough desiccation. The tubes were then sealed off with a Hoke gas-oxygen torch (FIGS. 2, 3).

While we have no evidence that the viability of cultures dried at pressures of 1 to 2 mm. of mercury is not satisfactory, drying

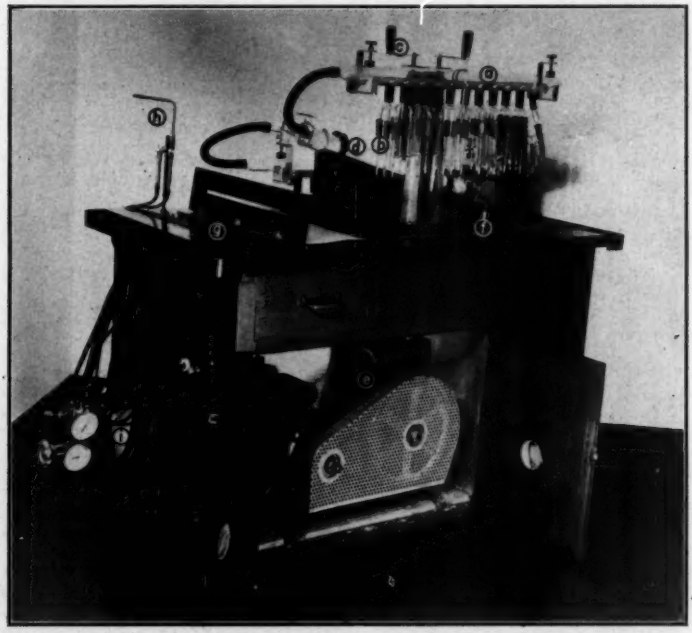

FIG. 2. Portable lyophil apparatus, a, manifold; b, lyophil preparations in final stage of drying; c, screw lift for raising and lowering manifold; d, drierite column; e, vacuum pump; f, freezing bath; g, multiple terminal panel for testing evacuation of finished preparations; h, oxygen-gas torch; i, oxygen storage tank.

proceeds more favorably when a vacuum between 200 and 500 μ of mercury is maintained.

Twenty-four hours after processing, the lyophil preparations were tested with a high-frequency, spark-coil tester to ascertain the existence of a vacuum in the completed cultures. Any tube which failed to show evidence of a good vacuum was discarded. The four finished preparations of each strain were then placed in

a small, screw-cap vial and were stored in a refrigerator at 3° to 5° C. One of the advantages of lyophil preservations is the saving of storage space; for example, in our trays, which measure 14½" × 12½" × 2½", quadruplicate preparation of 300 separate cultures can be stored.

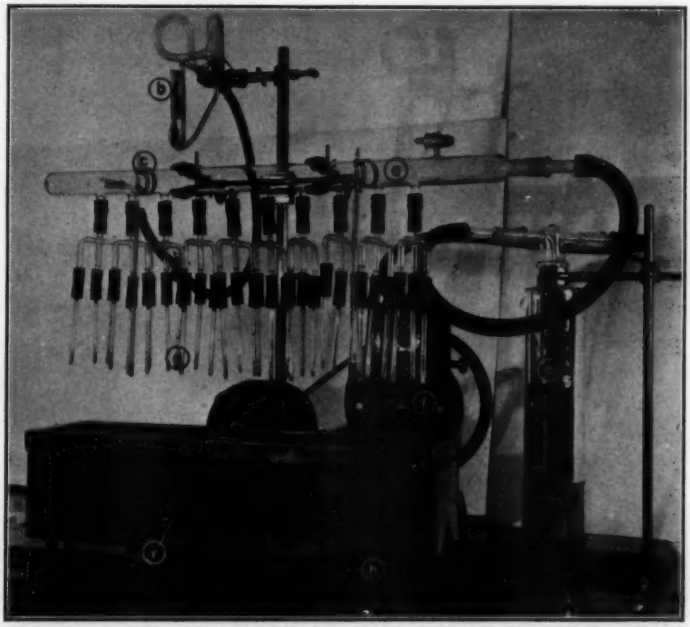

FIG. 3. Lyophil apparatus, table model. a, manifold; b, Bruner-type vacuum gauge; c, thermometer; d, lyophil preparations in final stages of desiccation; e, Dewar flask containing a water-vapor trap immersed in CO_2-ice and methyl cellosolve; f, vacuum pump; g, insulated freezing bath; h, vacuum tester; i, oxygen-gas torch.

The large apparatus shown in figure 2 possesses several marked advantages over the smaller machine of Wickerham and Andreasen (**1942**): (1) the screw-lifting and lowering device (FIG. 2 C) simplifies temperature control during drying; (2) the apparatus is a portable unit which can be easily moved from one laboratory to another; (3) a stationary pressure gauge makes it possible to check the amount of vacuum during processing; (4) a multiple

terminal panel facilitates the testing of finished lyophilized preparations for the presence of adequate vacuum; and (5) 60 lyophil preparations can be made in one operation.

Besides these advantages, the apparatus under discussion has certain disadvantages. The large number of rubber connections makes it difficult to attain a high degree of vacuum, and the $CaSO_4$ (Drierite) used as a desiccant must be frequently regenerated or replaced. With these two limitations in mind, a third model (FIG. 3) was constructed which has proved highly satisfactory. A single manifold with only 30 outlets is used, and ground glass joints have been inserted as connections wherever this is feasible to further reduce sources of possible leaks. The manifold is supported on a horizontal bar attached to a sliding collar on an upright standard. It is raised and lowered manually and can be clamped into any desired position by means of a bolt with a wing nut. The Drierite column has been replaced by a water-vapor trap which is immersed in a bath of solid CO_2 and methyl cellosolve and can be emptied and reassembled within a few minutes.

In recent months, we have used a modified technique suggested by Dr. Wickerham, involving the continuous immersion of the lyophil tubes during the drying process. The preparations are first submerged in a bath at approximately — 30° to — 40° C., and then sufficient cellosolve at 0° C. is added to raise the temperature to approximately — 10° C. The bath is allowed to warm up gradually but is not permitted to go above 0° C. until the preparations appear completely dry. Finally, the tubes are raised to room temperature, and drying is continued for an additional one-half hour.

To recultivate material preserved in lyophil form, the tube is marked with a file scratch, wiped off with alcohol or some other disinfectant, and broken open. The pellet is then dissolved in a small amount of sterile water or nutrient broth, and the resulting suspension is streaked on a suitable medium and incubated at room temperature for a period of a few days, or until typical colonies develop. New isolations are then made, and the culture may be continued in agar slants or relyophilized within 2 or 3 weeks. Resuspended spores from lyophil preparations may be used di-

rectly for the inoculation of flasks or other small vessels employed in actual fermentation investigations.

VIABILITY TESTS OF SELECTED CULTURES

Using the method described by Wickerham and Andreasen (**1942**) and an apparatus similar to that employed by them, a group of 34 selected cultures were preserved in lyophil form during the spring and early summer of 1941. These cultures can be roughly divided into three groups, namely: Group I: Cultures of industrial importance, including citric acid-producing strains of *Aspergillus niger* (NRRL *67, 584, 599,* and *602*); gluconic acid-producing strains of *A. niger* (NRRL *3* and *67*) and *Penicillium chrysogenum* (NRRL *811*); diastatic enzyme-producing strains of *A. flavus* (NRRL *693*), *A. Oryzae* (NRRL *692*), and *Rhizopus delemar* (NRRL *1472*); an itaconic acid-producing strain of *A. terreus* (NRRL *265*); fumaric acid-producing strains of *R. Oryzae* (NRRL *1526* and *1528*); and a d-lactic acid-producing strain of the same species (NRRL *395*). Two strains of the penicillin-producing culture of *P. notatum* isolated by Fleming (NRRL *824* and *1209*) were processed a few months later. Group II: Cultures difficult to maintain by conventional methods of periodic recultivation on agar slants, including such forms as *Mucor Rouxianus* (NRRL *1429*), *Phycomyces Blakesleeanus* (NRRL *1554* and *1555*), *Blakeslea trispora* (NRRL *1718*) and *Aspergillus itaconicus* (NRRL *161*). Group III: Cultures characterized by particularly striking morphological and cultural characteristics, including such forms as *Penicillium claviforme* (NRRL *1002*), *P. islandicum* (NRRL *1038*), *P. vinaceum* (NRRL *739*), a tan-spored mutant of *Aspergillus niger* (No. P-88 B), and others.

For the first group of cultures, the objective was twofold: to determine (1) if industrially important cultures could be preserved by quick freezing and thorough vacuum desiccation, and (2) if cultures so preserved would retain, unaltered, the physiological properties which render them valuable. For the second group, the primary objective was to determine if the period of viability could be prolonged materially, which would increase the usefulness of these forms by reducing the work necessary to keep them viable. With the third group the objective was to determine

TABLE 1

VIABILITY OF LYOPHILIZED PREPARATIONS OF SELECTED MOLD CULTURES TESTED AT INTERVALS UP TO APPROXIMATELY 40 MONTHS

Name	NRRL No.	Date processed	Viability of lyophilized cultures							
			Test No. 1		Test No. 2		Test No. 3		Test No. 4	
			Age in mo.	Viability	Age in mo.	Viability	Age in mo.	Viability	Age in mo.	Viability
Aspergillus flavus Link	484	5/ 5/41	4	+++[a]	26	+++	32½	+++	41	+++
A. flavus Link	693	6/25/41	2	+++	24½	+++	30½	++++	38½	+++
A. itaconicus Kinoshita	161	5/14/41	3½	++	26	++	33½	+	41	+
A. niger van Tieghem	3	5/ 5/41	4	+++	26	+++	32½	++++	41	+++
A. niger van Tieghem	67	5/29/41	3	+++	25½	+++	32½	+++	40½	++
A. niger van Tieghem	328	6/ 4/41	3	++++	25	++++	31½	++++	39½	++++
A. niger van Tieghem	334	5/29/41			25½	++	31½	++	39½	+++
A. niger van Tieghem	584	5/29/41			25½	+++	31½	+++	39½	+++
A. niger van Tieghem	599	5/28/41			25½	+	31½	+	39½	+
A. niger van Tieghem	602	5/29/41	3	+++	25½	+++			39½	+++
A. niger [tan mutant]	P-88B	4/ 8/41	4½	+++	18	++++				
A. Oryzae (Ahlburg) Cohn	692	6/25/41			24½	++++	30½	++++	38½	++++
A. Oryzae (Ahlburg) Cohn	695	6/25/41			24½	++++	30½	++++	38½	++++
A. Sydowi (B. & S.) Th. & Ch.	P-35	6/20/41	2½	++++	24½	++++			38½	++++
A. terreus Thom	265	6/25/41	2	++++	24½	++++	31½	++++	38½	++++
A. terreus Thom	273	6/ 4/41	3	++++	25	++++			39½	++++
Penicillium chrysogenum Thom	811	5/ 7/41	3½	++++	26	++++	32½	++++	40½	++++
P. claviforme Bainier	1002	6/21/41	2½	++++	24½	++++			40	+++
P. islandicum Sopp	1038	6/25/41	2	++++	24½	++++			38½	++++
P. lemoni Sopp	1042	6/25/41	2	++++	24½	++++			38½	++++
P. notatum Westling	1209	10/17/41			20½	++++	26½	++++	36	++++
P. notatum Westling	824	1/ 6/42			18	++++	24	++++	34	++++
P. purpurogenum var. *rubri-sclerotium* Thom	1064	5/ 4/41	4	+++	26	+++	31½	+++	40	+++
P. vinaceum Gilman and Abbott	739	6/21/41	2½	++	24½	+			38½	+
Gliocladium vermoeseni (Biou.) Thom	1752	6/25/41	2	+++	24½	+++			38½	+++
Blakeslea trispora Thaxter	1718	6/19/41	2½	+++	24½	+++	31	+++	39	+++
Mucor Ramannianus Möller	1559	6/15/41	2½	++++	24½	++++	31	++++	39	++++
Mucor Rouxianus (Calmette) Wehmer	1429	6/19/41	2½	+++	24½	+++	31	+++	39	+++
Phycomyces Blakesleeanus (+) Burgeff	1554	6/19/41	2½	+++	24½	+++	31	+++	39	+++
Phyco. Blakesleeanus (−) Burgeff	1555	6/19/41	2½	+++	24½	+++	31	+++	39	+++
Rhizopus delemar (Boid.) Weh. & Hanz.	1472	6/18/41	2½	+++	24½	+++	31	+++	38½	+++
Rhizopus Oryzae Went. & Pr. Geerl.	395	5/ 6/41	4	+++	26	++	32½	+++	40½	+++
Rhizopus Oryzae Went. & Pr. Geerl.	1526	5/ 7/41	4	+++	26	++	32½	+++	40	+++
Rhizopus Oryzae Went. & Pr. Geerl.	1528	5/29/41	3	+++	25½	++	31½	+++	39½	+++

[a] ++++ = excellent viability +++ = good viability ++ = fair viability + = poor viability

if forms preserved by the lyophil method would retain, unchanged, the distinctive morphological characters which distinguish them.

Results obtained in viability tests with these selected cultures are presented in table 1. The first preparations were opened when 2 to 4 months old. Growth was obtained in every case, and, without exception, the resulting cultures were entirely typical of the strains under observation. A limited number of preparations, not recorded in the table, were opened at 5 to 6 months. These likewise appeared wholly normal. It was at this time (December, 1941) that we embarked upon the mass processing of the entire mold collection. With few exceptions, a complete set of the 34 cultures selected for preliminary study was tested for viability at approximately 2 years, again at approximately 31 months, and lastly at approximately 40 months. Growth has been obtained in every case, although in some instances this has been only fair, and in a few cases definitely poor (TABLE 1). In the cases of *A. itaconicus* and *P. vinaceum,* limited growth may have resulted from the low concentration of spores contained in the initial preparations (verified by microscopic examination). In *A. niger,* NRRL *599,* abundant spores were present, and limited growth may have resulted from some factor such as the age of the spores or the degree of final dryness. Subsequent tests with this strain (TABLE 2) failed to substantiate an early belief that certain strains of *A. niger* could not be processed satisfactorily.

No attempt was made to measure quantitatively the number of viable spores in any of the preparations reported in table 1. Any cultures scored "++" (fair) or better, however, could be considered satisfactory from the standpoint of maintaining stock cultures, since such a rating implied the development of a reasonable number of colonies within a period of 3 to 4 days. Only in the case of *Aspergillus niger,* NRRL *599, A. itaconicus* (NRRL *161*), and *P. vinaceum* (NRRL *739*) were viabilities dangerously low. The few colonies which developed in each of these cases, however, were entirely characteristic of the strains under observation. It is questionable how much longer parallel lyophil preparations of these strains can be depended upon as reliable stock sources, although there has not been any marked reduction in the number of

colonies developing from progressively older preparations opened during the course of the investigation (TABLE 1).

In all cases where comparative tests have been made, the physiological characteristics of cultures preserved in lyophil form have duplicated those of the same strains preserved in agar slant cultures under optimum conditions. Specific examples will be subsequently noted (pp. 517 to 523).

A comparison of viability of certain of these strains (TABLE 1) in agar slant cultures and in lyophil preparations is of special interest. It is regrettable that we did not retain agar slants of all of the selected strains of the same age as the dried preparations.[6] We believe, however, that enough have been retained to permit a limited comparison and evaluation of the two methods. Agar slant cultures of *A. niger,* NRRL *3* and *67,* yielded viable cultures after 40 and 42 months, respectively; NRRL *602* was viable after 31½ months; whereas NRRL *328, 584,* and *599* failed to grow at 25 months. *Aspergillus flavus,* NRRL *484,* was viable at 40 months; *A. terreus,* NRRL *273,* at 32 months, and NRRL *265* at 30 months. *Aspergillus itaconicus* failed to grow at 40 months and grew very poorly at 28 months. *Penicillium vinaceum, P. purpurogenum* var. *rubri-sclerotium,* and *P. chrysogenum* were viable at 40 months. *P. notatum* (NRRL *824*) showed good growth up to 34 months. *Rhizopus delemar* failed to grow at 15 or 20 months. *R. Oryzae,* NRRL *1528,* failed to grow at 23 months and above, while a second strain of *R. Oryzae,* NRRL *1526,* yielded negative results from a tube culture of 15 months, positive from one of 20 months, and again negative from a still older tube culture. Viable cultures were obtained from tubes of *R. Oryzae,* NRRL *395,* up to 30 months. All agar slant cultures had been continuously stored in a refrigerator at 3° to 5° C. Neither the agar slant cultures nor the lyophil preparations were sufficiently old to justify more than tentative conclusions, but more consistently positive results were obtained from the lyophilized cultures. Results obtained with *Rhizopus Oryzae,* NRRL *1526,* are of particular interest since they illustrate that age alone

[6] Our usual procedure is to retain old agar slants for periods of 6 months to a year after new transplants are made. In exceptional cases they may be retained for longer periods.

is not the determining factor governing viability, but that conditions developing within the individual culture tube are probably responsible. The same conclusion is suggested by the behavior of the several strains of *Aspergillus niger*. In no case did cultures developing from old agar slants appear superior to those developing from lyophil preparations, and it is our considered opinion that the latter method will prove more satisfactory for the preservation of mold cultures in stable form over long periods of time. As is noted in the discussion (p. 523), the lyophil technique possesses certain outstanding advantages quite aside from the anticipated, but still only partially confirmed, extension of viability.

FURTHER VIABILITY TESTS

During the period from January to June 1942, quadruplicate lyophil preparations were made of each of the 1850 different mold cultures which were at that time contained in the Culture Collection. When these preparations were from 19 to 23½ months old, tubes of 140 selected strains representing 128 different species in 44 genera were tested for viability, and the resulting colonies were examined culturally and microscopically. In conducting this survey, an effort was made to include a wide variety of forms in order that the results would provide a reliable measure of the practicality of the lyophil technique as a means of preserving molds. Typical species from all of the major groups of the *Aspergilli* and *Penicillia* were selected, as were also representative species and genera of the Mucorales. In addition, 2 members of the Entomophthorales and 23 miscellaneous forms, representing as many different genera, were included.

The results of this survey are presented in table 2.

Aspergilli: Viable cultures were obtained from each of the strains of *Aspergilli* tested, and, of the 41 strains representing 37 different species, only 5 failed to show good or excellent viability and growth. In every case, the resulting colonies were entirely typical. Microscopic examinations showed conidial heads and other structures to be characteristic of the strains under observation.

The five cultures failing to show good or excellent viability included a strain of *Aspergillus carbonarius* (NRRL *369*), a large-

TABLE 2

VIABILITY OF MOLD CULTURES PRESERVED IN LYOPHIL FORM FOR PERIODS OF FROM 1½ TO 2 YEARS

Name	NRRL No.	Age in months	Viability
Aspergillus clavatus Desm.	4	20½	++++[a]
A. giganteus Wehmer	10	20½	++++
A. repens (Cda.) DeBary	17	20½	++++
A. ruber (Brem.) Thom & Raper	55	20½	++
A. Chevalieri (Mang.) Thom & Church	79	20½	+++
A. amstelodami (Mang.) Thom & Raper	94	20	++++
A. umbrosus Bain. & Sart.	121	21	++++
A. niveo-glaucus Thom & Raper	127	21	+
A. echinulatus (Delacr.) Th. & Ch.	131	21	+
A. restrictus Smith	148	21	++++
A. itaconicus Kinoshita	161	20½	+++
A. fumigatus Fresenius	163	21	++++
A. Fischeri Wehmer	181	21	++++
A. nidulans (Eidam) Wint.	187	21	++++
A. rugulosus Thom & Raper	211	21	++++
A. variecolor (B. & Br.) Th. & Rap.	214	21	++++
A. unguis (Em.-Weil & Gaud.) Th. & Rap.	216	21	++++
A. versicolor (Vuill.) Tiraboschi	226	21	++++
A. Sydowi (Bain. & Sart.) Th. & Ch.	247	21½	++++
A. terreus Thom	255	21½	++++
A. ustus (Bain.) Thom & Church	275	18½	++++
A. flavipes (Bain. & Sart.) Th. & Ch.	286	21	++++
A. candidus Link	308	21½	++++
A. alliaceus Thom & Church	315	21½	++++
A. niger v. Tieghem	319	22	++++
A. niger v. Tieghem	322	22	++++
A. niger v. Tieghem	334	22	++++
A. niger v. Tieghem	566	23½	++++
A. niger v. Tieghem	599	23½	++++
A. cinnamomeus Schiem.	348	21½	++++
A. carbonarius (Bain.) Thom	369	21½	++
A. Wentii Wehmer	377	22	++++
A. quercinus (Bain.) Thom & Church	387	22	++
A. ochraceus Wilhelm	398	22	++++
A. ostianus Wehmer	420	22	++++
A. terricola var. *americana* Marchal	424	22	++++
A. tamarii Kita	427	22	++++
A. luteo-virescens Blochwitz	444	22	++++
A. Oryzae (Ahlburg) Cohn	447	23	++++
A. flavus Link	482	23	+++
A. parasiticus Speare	502	23	++++
Penicillium Thomii Maire	701	22½	++++
P. javanicum v. Beyma	707	22½	++++
P. spinulosum Thom	723	22½	++++
P. roseo-maculatum Biourge	729	22½	++++
P. carmino-violaceum Dierckx	733	22½	++++
P. vinaceum Gilman & Abbott	739	22½	++
P. decumbens Thom	741	22½	++++
P. lividum Westling	754	22½	++++
P. implicatum Biourge	763	23½	++++
P. baiiolum Biourge	772	23½	++++
P. Waksmani Zal.	778	23½	++++
P. digitatum Sacc.	786	23½	++++

TABLE 2—(*Continued*)

Name	NRRL No.	Age in months	Viability
P. oxalicum Currie & Thom	789	23½	++++
P. citrinum Thom	805	24	++++
P. chrysogenum Thom	807	24	++++
P. notatum Westling	824	24	++++
P. baculatum Westling	843	24	++++
P. Melinii Thom	847	23½	++++
P. puberulum Bainier	845	23½	++++
P. roqueforti Thom	849	23½	++++
P. stoloniferum Thom	859	23½	++++
P. ochraceum (Bainier) Thom	869	23½	++++
P. camemberti Thom	877	22½	++++
P. biforme Thom	886	21	++++
P. lilacinum Thom	895	21	++++
P. janthinellum Biourge	905	21	++++
P. nigricans Bainier	915	21	++++
P. terrestre Jensen	933	21	++++
P. cyclopium Westling	941	20	++++
P. viridicatum Westling	963	20	++++
P. expansum (Link) Thom	976	20	++++
P. italicum Wehmer	983	20	++++
P. Urticae Bainier	989	20	++++
P. patulum Bainier	994	20	++++
P. claviforme Bainier	1002	20	++++
P. duclauxi Delacr.	1030	20	++++
P. islandicum Sopp	1037	20	++++
P. herquei Bainier & Sartory	1040	20	++++
P. rugulosum Thom	1045	20	++++
P. purpurogenum Stoll	1061	20½	++++
Gliocladium deliquescens Sopp	1086	20	++++
Glio. fimbriatum Gil. & Abb.	1090	20	++++
Glio. roseum Bainier	1084	20	++++
Paecilomyces Varioti Bainier	1122	20	++++
Scopulariopsis brevicaulis (Sacc.) Bain.	1101	20	++++
Scop. brevicaulis var. *glabrum* Thom	1109	19	++++
Absidia coerulea (−) Bainier	1310	22	++++
Absidia coerulea (+) Bainier	1314	22	++++
Circinella spinosa (−) v. T. & le Mon.	1356	22	+++
Circinella spinosa (+) v. T. & le Mon.	1359	22	+++
Chaetocladium Brefeldii v. T. & le Mon	1349	22	+++
Coemansia pectinata Bainier	1564	19½	++
Cunninghamella echinulata (+) (Thax.) Sacc.	1382	22	+++
Cunninghamella echinulata (−) (Thax.) Sacc.	1384	22	+++
Helicostylum sp. (+)	1396	21½	+++
Mucor genevensis Lendner	1412	21½	++++
Mucor hiemalis (−) Wehmer	1419	21½	++++
Mucor mucedo (+) Bref.	1424	21½	++++
Mucor mucedo (−) Bref.	1425	21½	++++
Mucor Rouxianus (Calmette) Wehmer	1429	22	++
Mycotypha microspora Fenner	684	21½	++++
Parasitella simplex (−) Bainier	1459	22	++++
Phycomyces Blakesleeanus (−) Burgeff	1464	21½	+++
Phycomyces Blakesleeanus (+) Burgeff	1465	21½	++
Rhizopus arrhizus Fischer	1470	21½	++++
Rhizopus nodosus Namysl.	1474	21½	++++

TABLE 2—(*Continued*)

Name	NRRL No.	Age in months	Viability
Rhizopus nigricans (+) Ehrenberg	1477	21½	++++
Rhizopus nigricans (−) Ehrenberg	1478	21½	++++
Rhizopus nigricans (−) Ehrenberg	1483	21½	++++
Rhizopus nigricans (+) Ehrenberg	1484	21½	++++
Syncephalastrum sp. (−)	1485	21½	++++
Thamnidium elegans Link	1613	19½	+++
Zygorhynchus heterogamus (±) Vuill.	1489	21½	+
Conidiobolus sp.	1612	19½	−
Entomophthora apiculata Thaxter	1626	19½	−
Botryosporium sp.	1710	19	++++
Botrytis sp.	1285	19½	−
Botrytis spectabilis	1776	19½	++++
Cadophora Richardsiae Nannf.	1630	19½	++++
Catenularia fuliginea Saito	1299	19	++++
Ceratostomella adiposum (Butler) Sart.	1801	22	+++
Chaetomium globosum Kunze	1669	19	−
Cladosporium fulvum Cke.	1671	19	++++
Dipodascus uninucleatus Biggs	1629	19½	+++
Epidermophyton interdigitale (Pr.) MacCar.	1295	19	++++
Fusarium moniliforme Sheld.	1675	19	+
Gymnoascus sp.	1677	19	++
Helminthosporium sp.	1680	19	++
Metarrhizium sp.	1800	22	++++
Microascus trigonosporus Emm. & Dodge	1570	23½	+++
Monascus purpureus Went.	1596	19½	+++
Monilia sitophila (Mont.) Sacc.	1275	20½	++++
Sordaria fimicola (Rab.) Ces. & de Not.	1558	19½	+
Stachybotrys lobulata Berk.	1695	19	++++
Trichoderma Koningi Oud.	1761	21	++++
Trichothecium roseum Link	1588	19½	+++
Tritirachium dependens Limber	1210	19	++++
Verticillium albo-atrum Rein. & Berth.	1204	19½	++++

++++ = excellent viability
+++ = good viability
++ = fair viability
+ = poor viability
− = no growth

spored member of the *A. niger* group; *A. quercinus* (NRRL *387*), a light-sporing, heavy sclerotium-producing member of the *A. ochraceus* group; and *A. ruber* (NRRL *55*), *A. niveo-glaucus* (NRRL *127*), and *A. echinulatus* (NRRL *131*), all ascosporic members of the *A. glaucus* group.

Low viability in *A. quercinus* probably resulted from the limited number of conidia contained in the dried suspension, and the limited growth of *A. carbonarius* may have been due at least in part to the large dimensions of its conidia. Three of the five

strains showing limited viability belonged to the *A. glaucus* group, and two of these, *A. niveo-glaucus* and *A. echinulatus,* possessed large conidia and ascospores, suggesting the possibility that large-spored members of this group may not lend themselves well to preservation by the lyophil method. Small-spored species of the same group such as *A. Chevalieri, A. repens,* and *A. Amstelodami,* on the other hand, consistently showed excellent viability and growth. *A. niger,* NRRL *599,* which had shown poor viability in the tests of selected cultures reported above, showed excellent viability in the present series. Among five strains of *A. niger* included for the purpose of comparing different representatives of a single species, no apparent difference in viability was observed.

Penicillium and allied genera: Altogether 40 strains of *Penicillia* representing a like number of species were tested. Included were representatives of such well-known and widely discussed species as *P. notatum, P. chrysogenum, P. patulum, P. claviforme,* and *P. roqueforti.* Excellent viability was obtained from lyophil preparations of all species with the exception of *P. vinaceum,* in which growth was only fair (TABLE 2); as in the earlier tests (TABLE 1), limited growth is believed to have resulted more from a dearth of conidia in the lyophil preparation than from any marked inability of the culture to withstand the freezing-drying process. Subsequent microscopic examination of a duplicate lyophil preparation revealed the presence of comparatively few spores.

Lyophil preparations of six representative forms belonging to the related genera *Gliocladium, Scopulariopsis,* and *Paecilomyces* showed excellent viability and growth (TABLE 2).

In all cases, colonies developing from lyophil preparations appeared typical of the strains under observation. Microscopic examination provided confirmatory evidence.

Mucorales: Viability tests were made on 27 strains of the Mucorales representing 19 different species. Of this number, plus and minus strains were included for 6 species, and for the single species, *Rhizopus nigricans,* 2 plus and 2 minus strains were tested. Results are presented in table 2. Excellent or good growth was obtained in all cases with the exception of *Mucor Rouxianus,* NRRL *1429,* a strain of *Phycomyces Blakesleeanus* (+), NRRL *1465, Coemansia pectinata,* NRRL *1564,* and *Zygor-*

hynchus heterogamus, NRRL *1489.* In the last of these cultures viability and growth were poor, and examination of a second lyophil preparation from the original set of 4 revealed the presence of numerous zygospores but very few sporangiospores. Microscopic examination of duplicate preparations of *Phycomyces* and *Coemansia* revealed the presence of comparatively few spores.

Cultures developing from lyophil preparations of all of the Mucorales appeared typical.

Entomophthorales: Representatives of the Entomophthorales that were tested for viability have shown consistently negative results. Included in the present survey were strains of *Entomophthora apiculata,* NRRL *1626,* and *Conidiobolus* sp., NRRL *1612.* A second strain of *Conidiobolus,* NRRL *1255,* has been subsequently investigated. For each of these cultures a new series of lyophil preparations has been made, and viability tests have been conducted within a few days. In no case has growth been obtained. The method as employed by us is obviously not applicable to this group.

Miscellaneous genera: Viability tests were conducted on 23 cultures representing an equal number of genera, belonging mostly in the Fungi Imperfecti. Results are shown in table 2. Excellent to good viability was obtained in most cases, but negative results were obtained with *Chaetomium globosum,* NRRL *1669,* and *Botrytis* sp., NRRL *1285.* The first of two preparations of *Sordaria fimicola,* NRRL *1558,* was negative, whereas a duplicate tube yielded very few colonies. Duplicate lyophil preparations of each of the above-named cultures were subsequently examined microscopically; numerous hyphal fragments were observed, but no spores were found. This absence of spores possibly accounts for the inconsistent growth of *Sordaria* and for the lack of growth in *Chaetomium* and *Botrytis.* Such an explanation derives additional support from tests subsequently performed in which new lyophil preparations, containing ample spores, were made of these strains and were tested for viability. Results were positive. It should, of course, be borne in mind that in the latter tests no time element was involved, whereas the preparations found negative were already 19 months old.

Poor viability characterized a preparation of *Fusarium moniliforme,* NRRL *1675,* although a duplicate tube was found to contain abundant spores—no explanation is available. In recent tests, preparations of *Fusarium bulbigenum* var. *Lycopersici,* NRRL *1985,* have shown excellent viability when tested immediately after being processed.

PRESERVATION OF PENICILLIN-PRODUCING CULTURES

In the investigation of any fermentation, the importance of maintaining cultures in a stable and highly productive state cannot be overemphasized. In the production of penicillin, the problem of culture preservation assumes special significance because of the marked tendency for highly productive strains to vary, or mutate, when frequently transferred, or when grown upon nutrient-rich substrata (Raper and Alexander, **1945**). The need for a simple and satisfactory method of maintaining stock cultures was recognized early in the work on penicillin. Since the autumn of 1941, multiple lyophil preparations (often running up to 30 to 60 tubes) have been made of cultures which seemed to offer particular promise.

As our investigations have progressed, it has frequently been desirable to compare penicillin production by strains in current use with those employed in earlier experiments. In a number of cases, such comparisons have been made between fresh stock cultures and cultures of the same strains from lyophil preparations of varying age up to 40 months. In making these comparisons, the culture medium and methods reported by Raper, Alexander, and Coghill (**1944**) were employed. Results obtained with several series of such cultures are presented in table 3. Paired entries represent parallel cultures of the same strain, derived in the one case from the recultivation of a lyophil preparation, in the other case by transfer from the stock culture. The term "stock culture," [7] refers to agar slant cultures maintained in duplicate in

[7] In the case of the *Penicillia,* such stock cultures are regularly transplanted on Czapek's solution agar, allowed to grow at room temperature for a period of 2 to 3 weeks, and then stored in a refrigerator at 3° to 5° C. for a period up to 8 months, during which time no further growth occurs, and the cultures remain in a dormant state.

our permanent collection under conditions believed to be optimum for preserving the mold in a stable and viable form. Subcultures seeded from current stocks of various strains of *P. notatum* and *P. chrysogenum* are employed in our work, and, when transplanted upon sporulation media (Moyer and Coghill, **1945**), grow rapidly and produce a heavy and uniform sporulation. At the same time, it has been our consistent experience that lyophilized cultures of these same strains planted upon similar media produced uniform and abundant crops of spores equal in all respects to plates seeded from the agar stock cultures. This being true, it has been possible to inoculate surface production flasks with equal and like quantities of spores developed from these two different sources.

Similarities or differences in yields of penicillin, pH, and dry weights of the mycelia can be interpreted as resulting from similarities or differences existing between the strains maintained by these different methods of preservation, since in any pair of cultures the substrate was of exactly the same composition, the temperature of incubation was constant, and inoculations were as nearly uniform as it was possible to make them.

Examination of the results presented in table 3 clearly indicates that cultures of *P. notatum* and *P. chrysogenum* can be preserved by the lyophil technique for periods up to 3 years without loss of their capacity to produce penicillin. In general, penicillin yields and pH values agreed quite closely in the parallel cultures from day to day; and in 9 of 13 cases, the cultures seeded from the lyophil source actually showed slightly higher average yields of penicillin than those seeded from stock cultures grown upon agar. This difference, however, is not marked, and it is probably not significant. In every case investigated, the mycelial mats developed from lyophil "seed" and those from stock "seed" appeared strikingly similar. In the majority of cases, this cultural similarity was likewise reflected in the approximately equal weights of the dried mycelial mats harvested from individual flasks (TABLE 3).

While comparative data need not be presented, it should be noted that selected strains maintained in lyophil form have retained unaltered their capacity to produce penicillin in submerged culture.

TABLE 3

TABLE 3

PENICILLIN PRODUCTION IN SURFACE CULTURE BY SELECTED STRAINS OF PENICILLIUM NOTATUM WESTLING & P. CHRYSOGENUM THOM (1) PRESERVED IN LYOPHIL FORM AND (2) MAINTAINED UPON CZAPEK'S SOLUTION AGAR SLANTS UNDER OPTIMUM CONDITIONS

Name	NRRL no.	Type of culture	Age in mos.	Penicillin production								Mat weights in grams		
				Penicillin yields										
				4th Day		5th Day		6th Day		7th Day		Flasks		
				pH	units[a]	pH	units	pH	units	pH	units	A	B	C
P. chrysogenum	807[b]	Lyo.	24	7.0	29	7.6	53	7.9	59	8.0	53	.926	.931	.982
P. chrysogenum	807	Stock[c]		6.9	25	7.5	45	7.9	56	7.8	50	1.004	.953	.942
P. chrysogenum	811	Lyo.	32	6.5	25	7.3	50	7.8	67	8.0	63	1.010	1.033	1.071
P. chrysogenum	811	Stock		6.3	25	7.1	45	7.6	60	8.0	59	.956	.979	1.018
P. chrysogenum	811	Lyo.	40	7.3	44	7.6	78	7.9	85	8.3	76	.911	.909	1.053
P. chrysogenum	811	Stock		7.1	43	7.4	73	7.8	83	8.3	90	.914	.943	1.027
P. notatum	824[d]	Lyo.	22	7.6	78	7.8	88	8.1	80	8.3	58	.941	.958	.964
P. notatum	824	Stock		7.4	78	8.0	82	8.1	74	8.3	62	.945	.949	.965
P. notatum	824	Lyo.	32	7.3	78	7.6	98	7.9	92	8.2	79	.843	.855	.877
P. notatum	824	Stock		7.5	78	7.8	90	8.1	81	8.4	40	.705	.663	.718
P. notatum	832[e]	Lyo.	24	7.6	53	7.9	70	8.1	52	8.4	26	.920	.929	.957
P. notatum	832	Stock		7.5	45	7.9	64	8.1	56	8.4	32	.869	.946	.941

[a] Yields of penicillin are expressed in Oxford units per milliliter.
[b] Type strain of *P. chrysogenum* Thom (Thom's No. 26).
[c] The "stock" cultures employed were current stocks, maintained on agar, from the permanent mold collection. They varied in age from 2 or 3 weeks to 5 or 6 months.
[d] The unimproved Fleming strain.
[e] Strain commonly employed for the production of penicillin in submerged culture.

TABLE 3—(*Continued*)

Name	NRRL no.	Type of culture	Age in mos.	Penicillin production								Mat weights in grams		
				Penicillin yields										
				4th Day		5th Day		6th Day		7th Day		Flasks		
				pH	units[a]	pH	units	pH	units	pH	units	A	B	C
P. notatum	1209[f]	Lyo.	25	7.6	75	7.9	85	8.0	68	8.3	43	.921	.935	.937
P. notatum	1209	Stock		7.3	75	7.6	65	8.1	68	8.3	45	.952	1.172	.968
P. notatum	1209	Lyo.	35	7.6	76	7.8	92	8.1	88	8.3	68	.823	.771	.970
P. notatum	1209	Stock		7.5	78	7.8	100	8.1	106	8.3	73	.759	.745	.813
P. notatum	1248	Lyo.	20	7.4	80	7.9	72	8.0	76	8.4	43	.970	.974	.971
P. notatum	1248	Stock		7.5	85	7.9	90	8.1	72	8.4	39	.980	.953	.962
P. notatum	1249	Lyo.	19	7.0	91	7.8	103	7.8	82	8.2	60	.776	.797	.807
P. notatum	1249	Stock		7.1	98	7.6	103	7.8	82	8.1	74	.792	.784	.801
P. notatum	1249	Lyo.	32	7.3	92	7.5	120	7.9	114	8.2	103	.758	.747	.847
P. notatum	1249	Stock		7.3	76	7.6	88	7.9	90	8.2	96	.905	.893	.937
P. notatum	1249.B21[g]	Lyo.	11	6.8	90	7.5	125	8.0	130	8.3	72	.766	.782	.844
P. notatum	1249.B21	Stock		7.0	92	7.7	115	8.1	111	8.3	63	.835	.851	.901
P. notatum	1249.B21	Lyo.	20	6.8	101	7.3	150	7.7	162	8.1	162	.787	.746	.831
P. notatum	1249.B21	Stock		6.8	98	7.2	137	7.7	159	8.1	148	.793	.788	.863

[f] Fleming strain as investigated by Drs. Florey, Heatley et al. in 1940–1941.
[g] Strain generally used for the production of penicillin in surface culture. Descended from the Fleming isolate; an improved strain developed at the Northern Regional Research Laboratory.

In some cases, as many as 60 replicate lyophil preparations have been prepared from a single good penicillin-producing culture, and during the ensuing months individual tubes have been opened from time to time to furnish inoculum comparable to that used in earlier experiments. In no case have we observed any decrease in the apparent viability of such cultures, as progressively older tubes have been opened, nor have we detected any reduction in their capacity to produce penicillin, although some of these are now more than 2½ years old. Lyophil preparations of *P. notatum*, NRRL *1249.B21* (the strain generally employed in industry for the surface production of penicillin), in particular, have been opened and tested frequently since this strain was first isolated. Where these preparations have been employed, penicillin yields have varied from experiment to experiment, just as they do when flasks are seeded from agar stock cultures, but no consistent change has been observed. It is our belief, therefore, that the lyophil technique provides an excellent means of preserving selected penicillin-producing cultures in unaltered form over long periods.

DISCUSSION

Although tests have not been in progress long enough to evaluate thoroughly the lyophil technique as a means of extending the viability of molds, much evidence is accumulating which indicates that this objective will, in all probability, be realized. Many strains have now been maintained in desiccated form for approximately 3½ years. In most cases there has been no apparent decrease in viability in the successively older preparations that have been examined, and in no instance has there been a marked decrease in viability with increased time. Furthermore, all cultures grown from lyophil preparations have appeared wholly typical of the strains under observation in both colony characteristics and in structural details.

The present investigations have been conducted on an extensive scale, and it now appears probable that most of the molds producing comparatively small aerial spores, or conidia, can be successfully preserved by the lyophil method. Excellent results have been obtained, almost without exception, with the *Penicillia* and closely allied forms. Most of the *Aspergilli* have been processed quite

satisfactorily, although the large-spored, ascosporic species of the *Aspergillus glaucus* group did not lend themselves well to this technique. Species of the Mucorales which produced abundant sporangiospores have been preserved satisfactorily, and tests completed up to the present time indicate that the period of viability of many members of this group is substantially extended by preservation in lyophil form. Consistently negative results have been obtained with the limited number of the Entomophthorales investigated. Positive, and, in most cases, satisfactory results were obtained with the Fungi Imperfecti. Strains of *Sordaria* and *Chaetomium,* which yielded negative results in initial tests, proved positive when new preparations containing abundant spores were made.

No attempts have been made to preserve any of the Myxogastrales or of the aquatic Phycomycetes. Spores of the Acrasiales, or pseudoplasmodium-forming slime molds, have been preserved with excellent results for periods up to 41 months, when last tested. Attempts to preserve the myxamoebae, or vegetative cells, of these same forms have been unsuccessful.

While many of the variations involved in the processing of large numbers of cultures have not been thoroughly investigated, certain general observations have been made which are believed significant. Cultures producing small spores in abundance have been preserved most satisfactorily. Cultures producing large spores have been preserved less successfully. Cultures producing large, highly organized spores, such as the primary conidia of *Conidiobolus* and *Entomophthora,* have failed to withstand the freezing-drying process. Preparations containing very few spores often yielded positive results, but preparations containing only vegetative mycelium in the form of hyphal fragments, and no specialized propagative bodies, regularly yielded negative results. It would appear that the presence of spores, conidia, or other definite propagative cells is essential for satisfactory lyophilization. Such cells should be present in abundance and the smaller their dimensions, the greater the probability that the species can be successfully preserved in desiccated form.

Not only do cultures preserved in lyophil form retain their distinctive cultural and morphological characteristics, but in all cases where tests have been made, physiological characteristics have like-

wise remained stable. Two examples will be cited. Cultures of penicillin-producing molds maintained in lyophil form for periods up to 40 months produce yields of penicillin equal to the same strains maintained in agar slant cultures under optimum conditions. In fact, lyophilized cultures have in many cases yielded slightly higher values than agar cultures, but it is doubtful whether the observed differences are significant. Itaconic acid-producing strains of *Aspergillus terreus* maintained in lyophil form for 40 months, when recultivated and tested, produced yields of itaconic acid [8] almost identical with those produced by cultures of the same strains recultivated four times and maintained for the same period on agar slants under optimum conditions. Brown (**1932**), Swift (**1937**), Elser, Thomas and Steffen (**1935**), Osterman and Rettger (**1941**), and others reported that bacteria so preserved retained their toxigenic and serological characteristics.

It is believed that the greatest usefulness of the lyophil technique as a means of preserving molds probably lies in the field of industrial fermentations. Using this method it is possible to make an unlimited number of dried preparations from a single and uniform suspension of spores taken from a selected culture or actual fermentation. Subsequent to this, one of these dried cultures can be opened and its contents recultivated whenever necessary with the assurance that the new growth will result from the spores originally processed. It is thus possible to set fermentations over an extended period with inoculum developed from an entirely uniform source. The possibility of contamination during storage is eliminated since the cultures are sealed in glass. It has been fully established in our studies that lyophilized cultures of *P. notatum,* NRRL *1249.B21,* and other high-yielding strains, retain at a high and stable level their capacity to produce penicillin. Replicate cultures from a single set of preparations have been opened from time to time over a period of almost 2 years, and uniformly satisfactory yields have been obtained. In fact, it is now our regular practice to check current stock cultures against such lyophilized preparations whenever any question arises regarding possible diminution in penicillin-producing capacity.

[8] The writers are indebted to Dr. L. B. Lockwood for making these comparisons.

SUMMARY

1. Almost all of the *Aspergilli* and *Penicillia* can be preserved in lyophil, or desiccated form. Such evacuated preparations, tested at intervals up to 40 months, generally showed no reduction in viability, and resulting cultures were entirely typical of the strains under observation.

2. Representative species of the Mucorales have been successfully preserved, and in the case of *Rhizopus, Phycomyces,* and other genera there is evidence of a marked extension of viability.

3. Attempts to preserve members of the Entomophthorales have been unsuccessful.

4. Representative Hyphomycetes were viable when tested at approximately 20 months.

5. Molds preserved in lyophil form apparently retain their biochemical and physiological characteristics in unaltered form. Strains of *Aspergillus terreus* preserved in this manner produced undiminished yields of itaconic acid after 40 months, while strains of *P. notatum* and *P. chrysogenum* retained at original levels their capacity to produce penicillin.

6. The lyophil technique provides a convenient means of preserving a large number of replicate cultures which can be used as seed material for standard cultures or to set series of fermentations over an extended period of time. Storage becomes a minor problem because of the small dimensions of the preparations. The possibility of contamination is eliminated during the storage period.

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